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Our goals can only be reached through a vehicle of a plan,

in which we must fervently believe, and upon which we must vigorously act

There is no other route to success (Pablo Picasso)

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Abstract

The aim of this study was to determine the influence of different winemaking techniques on phenolic composition and biological activities of musts and wines from grapes of Syrah and Cabernet sauvignon from two distinct Lebanese regions (Bekaa valley and Chouf district) and two consecutive vintages (2014 and 2015). Among these processes the impacts of pre-fermentative cold and heating maceration, enzymatic treatment, two different commercial yeast strains and fining agents were discussed in our study. Spectrophotometric and HPLC analysis of phenolic compounds showed that the pre-fermentative heating maceration leads to a better extraction of phenolic compounds than the pre-fermentative cold maceration. Tannins and total polyphenols extraction are favored by the temperature and the prolongation of maceration. Extraction of anthocyanins is also favored by the temperature with short duration since the extension of the maceration leads to a degradation of these compounds. Maceration enzymes addition at early stage of maceration, promoted higher concentration of total polyphenol and antioxidant activity compared to those macerated without added enzymes. Alcoholic fermentation results in a decrease of total polyphenols content which revealed differences between wines derived from X and Y strains. After alcoholic fermentation, almost all of the wine samples presented an increase of their percentage of inhibition with the occurrence of new types of biological activities which doesn‟t existed at must level. At the end, the results showed the importance of selecting a fining agent according to the type of wine and to minimize the dose of fining applied in order to conserve the content of phenolic compounds in wine.

Keywords: polyphenols, wine, oenological processes, antioxydant, fermentation, maceration

Résumé

Le but de cette étude était de déterminer l'influence des différentes techniques de vinification sur la composition phénolique et les activités biologiques des moûts et des vins issus de raisins de Syrah et de Cabernet sauvignon appartenant à deux régions libanaises distinctes (vallée de la Bekaa et la région de Chouf) et à deux millésimes consécutifs (2014 and 2015). Parmi ces procédés, les effets de la macération pré-fermentaire à froid et à chaud, du traitement enzymatique, de deux souches de levures commerciales et les agents de collage ont été discutés dans notre étude. L'analyse des composés phénoliques par spectrophotométrie et HPLC a montré que la macération pré-fermentaire à chaud entraine une meilleure extraction des composés phénoliques que la macération pré-fermentaire à froid. L‟extraction des tanins et des polyphénols totaux sont favorisés par la température et le prolongement de la macération. L‟extraction des anthocyanes est aussi favorisée par la température mais à courte durée puisque le prolongement de la macération entraine une dégradation de ces composés. Les moûts et les vins issus de l‟addition d‟enzymes pectolytiques au début de la phase de macération montrent des activités antioxydantes et des concentrations en polyphenols totaux plus élevées comparés à celles réalisées sans ajout d‟enzymes. La fermentation alcoolique provoque une diminution de la concentration des polyphénols totaux ce qui révèle des différences significatives entre les vins fermentés par les deux souches de levures X et Y. Après fermentation alcoolique, la quasi-totalité des échantillons de vin ont présenté une augmentation de leur pourcentage d'inhibition avec l'apparition de nouveaux types d'activités biologiques qui n'existait pas au niveau des moûts. A la fin, les résultats montrent l‟importance de bien choisir le type de colle selon le type de vin ainsi que de minimiser la dose de collage appliquée afin de conserver la teneur en composés phénoliques du vin.

Mots-clés: polyphénols, vin, procédés oenologiques, antioxydant, fermentation, macération

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Abbreviations

A431: Human Epithelial Carcinoma Cell line

A: Absorbance

ABA: Abscisic Acid

ABTS: 2, 2′-azinobis-3-ethylbenzothiazoline-6-sulfonic acid

Aβ: Amyloid-β Peptide

ACHE: Acetylcholinesterase

Acthi: Acetylthiocholine iodide

AF: Alcoholic fermentation

α-gluc: alpha glucosidase

AMPK: Adenosine Monophosphate-Activated Protein kinase

ANOVA: Analysis of Variance

ARE/Nrf2: Antioxidant Responsive Element/ Nuclear erythroid 2-related factor 2

B: Bentonite

BC: Before Christ

CA: Caffeic Acid

Cat: Catechin

C: Control

CD: Color Density

CHD: Coronary Heart Disease

ChE: Cholinesterase

CI: Color Intensity

CO_2: Carbon Dioxide

COX: Cyclooxygenase

CS-F: Cabernet Sauvignon Florentine

CS-ST: Cabernet Sauvignon Saint Thomas

Cy: Cyanidin

DMSO: Dimethyl Sulfoxide

DNA: Deoxyribonucleic Acid

DNS: Dinitrosalicylic acid

Dp: Delphinidin

DPPH: 2, 2-Diphenyl-1-Picrylhydrazyl

DTNB: 5, 5'-dithiobis-(2-nitrobenzoic acid)

EA: Egg Albumin

EEC: European Union Regulation

eNOS: endothelial Nitric Oxide Synthase

Epi: Epicatechin

Epig: Epicatechin gallate

EpiG, EGC: Epigallocatechin

FA: Ferulic acid

FR: Flash Release

FRAP: Ferric Reducing Ability of Plasma

GaHBr: Galanthamine Hydrobromide

GAE: Gallic Acid Equivalent

GA: Gallic Acid

(%G): Galloylation rate

G: Gelatin

glc: glycosylated

GLUT4: Glucose Transporter Type 4

GSPE: Grape Seed Proanthocyanidin Extract

h: hours

HCT116: Human Colon Cancer

HDL: High Density Lipoproteins

HDC: Histidine Decarboxylase

HFL-1: Human Foetal Lung Fibroblast

HPLC: High-Performance Liquid Chromatography

HSD: Honestly Significant Difference

IL6: Interleukin-6

$K_2S_2O_8$: Potassium Persulfate

LDL: Low Density Lipoproteins

LOX: Lipoxygenase

Mv: Malvidin

M: Mannoproteins

MCF7: Human Breast Cancer

MCP-1: Monocyte Chemoattractant Protein-1

mDP: Mean Degree of Polymerisation

MMP-9: Matrix Metallopeptidase 9

MOX: Micro-Oxygenation

mRNA: messenger Ribonucleic Acid

MTT: 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide

MW: Molecular Weight

$NaHSO_3$: Sodium Metabisulphite

NDGA: Nordihydroguaiaretic Acid

NF-KB: Nuclear Factor-KappaB

NO: Nitric Oxide

O_2: Oxygen

OD: Optical Density

OIV: International Organistaion of Vine and Wine

PCA: Principle Component Analysis

PC1: First Principal Component

PC2: Second Principle Component

PES: Polyethersulfone

pH: Potential of Hydrogen

PI3K: Phosphatidylinositol-4, 5-bisphosphate 3-Kinase

Pn: Peonidin

pNPG: 4-Nitrophenyl β-D-Glucuronide

Pro B1: Procyanidin B1

Pro B2: Procyanidin B2

Pvpp: Polyvinylpolypyrrolidone

Res: Resveratrol

ROS: Radical Oxygen Species

RP-HPLC: Reversed Phase High-Performance Liquid Chromatography

SB: *Saccharomyces bayanus*

SC: *Saccharomyces cerevisiae*

SD: Standard Deviation

SO_2: Sulfur Dioxide

Σ= Sum

SIRTI: Skinny Gene

Sy-F: Syrah-Florentine

Sy-ST: Syrah Saint Thomas

T: Tannins

TA: Total Anthocyanin

T/A: Tannins-Anthocyanins ratio

TP: Total Polyphenols

TPI: Total Polyphenol Index

UV-Vis: Ultraviolet-Visible

VEGF: Vascular Endothelial Growth Factor

VP: Vegetable Proteins

WHO: World Health Organization

XOD: Xanthine oxidase

YEPD: Yeast Extract Peptone Dextrose

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Introduction

The attribution of beneficial health effects to the consumption of wine goes back to the highest antiquity. However, wine has its detractors because of the harmful effects related to the presence of alcohol. Thus Hippocrates recommended wine to his patients, while Pythagores condemned it. This duality has persisted over time. In the late 1980s, the World Health Organization (WHO) highlighted the French Paradox, verifying the hypothesis that consumption of red wine at a reasonable dose (one or two glasses per day) has relatively lower incidence of coronary heart disease (CHD). Hence, the rate of cardiovascular mortality for the French people is lower than for their European neighbors. The anti-inflammatory, anticancer, antibacterial, antifungal, antiviral, neuroprotective, antiproliferative and antiangiogenic activities (Guilford and Pezzuto, 2011) of red wines are already known. These observations are not demonstrated for Lebanese red wines which have been little studied to date. Indeed, papers on the Lebanese wines, their phenolic composition and biological activities are rarely found in the literature.

Lebanese wine history begins with the Phoenicians and dates back more than five millennia. Later, in Roman times in the middle of the second century BC, a temple was dedicated to Bacchus, the god of wine, in the Baalbeck area. It was in the Bekaa valley that viticulture developed first. Modern history begins in 1857**,** when the Jesuit monks brought from Algeria Cinsault grapes. The Domaine des Tourelles was founded in 1868, followed by Nakad in 1923 and Musar in 1930. At the end of the 1975-1990 war, Ksara, Kefraya and Musar were the only known wines. Between 1997 and 1998 emerging areas such Wardy, Chateau St. Thomas, Heritage and Masaya were known.

Transformation of grape juice into wine is a complex process. The quality of wine obtained depends on such diverse factors as: raw material, oenological techniques employed, yeast strains… The quality of red wines is largely determined by the phenolic compounds, especially anthocyanins (responsible for the red color) and tannins (responsible for the sensation of astringency). The extraction of these compounds from the grape takes place mainly during the maceration phase. The conduct of the maceration depends mainly on the winemaker choices and should be regulated to favor the dissolution of the phenolic compounds to the maximum. However, the grape skin cell walls are limiting barrier that prevent the release of polyphenols

into the must during fermentation, for that reason 20 to 30% of the phenolic potential of the grape is found in wine. In order to improve the extractability of phenolic compounds, numerous technologies have been adopted such as pre-fermentation cold and hot macerations, pectolytic enzyme addition, flash release, thermovinification and carbonic maceration (Berger and Cottereau, 2000; Busse-Valverde et al., 2010). Besides, the chemical nature and the concentrations of phenolic compounds in wines are modulated by the raw material (grape variety, maturity ...) but also by the vinification conditions used (type and time of maceration, maceration enzymes added, yeast strains, fining agents, alcoholic and malolactic fermentation, filtration, ...).

This thesis is part of collaboration between the Chemical Engineering laboratory (LGC) and INPT (French partnerships) and the Lebanese Agricultural Research Institute (LARI) and Holy Spirit University of Kaslik (Lebanese partnerships). This work has been financially supported by LARI; most of the work has been done in Lebanon (LARI laboratory) except for the biological activities of wine analysis which has been done in the LGC laboratory. Grapes varieties were delivered by two Lebanese wineries: Clos St. Thomas and Chateau Florentine which are in constant search to improve quality. A thorough knowledge of their wines and the potentiality of their vineyards is today an indispensable approach.

The research work developed during this thesis is organized around three main objectives:

- Determination of the phenolic composition of musts obtained from the world-renowned grape varieties like Syrah and Cabernet Sauvignon. The purpose of this study was to determine the Lebanese terroir and vintage effects on the phenolic composition of wines respectively from two distinct regions (Chouf and west Bekaa) and two consecutive vintages (2014 and 2015).

- Determination of the impact of winemaking parameters on the phenolic composition and Biological activities of Lebanese wines. Among the parameters to be studied: i) the nature of maceration (pre-fermentation /cold, hot, with or without added enzymes) and the maceration time in order to determine kinetics of extraction of these phenolic

compounds and to define technical and optimum extraction time; ii) Impact of fermentation steps (alcoholic and malolactic fermentation) as well as the yeast strains used; iii) impact of some clarification techniques (fining agents).

The interest of this study was to introduce in the wine industry, the scientific knowledge allowing quality and safety improvement of the products as well as the productivity of the sector. This project is part of the developments in the Lebanese wine booming sector that might be both competitive and profitable by laying down quality and public health requirements. This project will also present practical knowledge to enologists regarding the winemaking processes in helping them to understand the interest and non-interest of certain techniques. After all, this knowledge will allow better management and profitability of the cellar by optimizing certain techniques such as maceration.

The manuscript is organized into five chapters

The first chapter includes a detailed literature on the different phenolic composition of grapes and wines, their impacts on human health and a review on the impact of winemaking processes on phenolic composition and content of wine.

The "Results and Discussion" section include chapters II, III and IV. Each chapter include an addition to the results and discussion a small introduction as well as material and methods detailing the progress of maceration, fermentation, clarification and the analysis of wines.

Chapter II entitled maceration steps is divided in two parts. The first study of part 1 sets out the effect of maceration time and temperature on the chromatic characteristics, flavonoids and non-flavonoids profile and biological activities of Syrah and Cabernet Sauvignon musts elaborated in two distinct Lebanese wine growing regions (Bekaa and Chouf district) using pre-fermentation cold (10°C) and heat maceration (60°C, 70°C and 80°C) compared to traditional winemaking (control, 25°C). The second study of this part show by means of statistical multivariate analyses (PCA) the terroir effects and define the best couple time/temperature of maceration for each

grape must giving more information for a correct planning and management of the winemaking operations in the Lebanese terroir. Part 2 exhibited firstly the influence of pectolytic enzyme addition and prefermentative heat maceration at different temperatures (60°C and 70°C and 70°C + enzymes) on the phenolic content and biological activities of Syrah and Cabernet Sauvignons red musts from two consecutive vintages (2014 and 2015) grown at Lebanese wine region (Bekaa valley, Saint Thomas) and secondly elucidate by means of statistical multivariate analyses (PCA) the vintage effects.

Chapter III presents the effect of two different commercial yeast strains (X and Y) on wine color, phenolic compounds and biological activities from two grape varieties musts (Syrah and Cabernet Sauvignon) from two distinct regions (Saint Thomas and Florentine) macerated at different temperatures (10°C, 60°C, 70°C and 80°C) from the 2014 vintage. As well as the effect of maceration enzymes on polyphenol composition of wines after alcoholic fermentation of Syrah and Cabernet Sauvignon Saint Thomas from the 2015 vintage premacerated at different temperatures with and without added enzymes (70°C, 70°C + enzymes) compared to the control (25°C) fermented by X and Y strains with and without enzymes.

Chapter IV exposes the effect of five different oenological fining agents (egg albumin, PVPP + casein, bentonite, gelatin and vegetable proteins) and two oenological additives (tannins and mannoproteins); as well as the study show the effect of different fining concentrations on the chromatic characteristics, phenolic composition, and antioxidant activity of Cabernet Sauvignon red wine from the 2014 vintage provided from Clos Saint Thomas.

Finally, the general Conclusions and the Perspectives will bring together the main findings as well as will explore future consideration in a subsequent study.

Chapter I. State of the Art

I.1. Grapes

The grape is the fruit of the cultivated vine (*Vitis vinifera and labrusca*). This is the second most cultivated fruit in the world. According to a report by the International Organization of Vine and Wine (OIV) about the world grape production (OIV, 2016), grapes production in 2015 is equivalent to nearly 76 million tons per annum. Figure I.1 shows the evolution of grapes production by country from 2000 till 2015. Growth in grapes production is particularly significant in China, USA, Chile and India. A decrease in production is noticed for Italy, France, Spain and Iran.

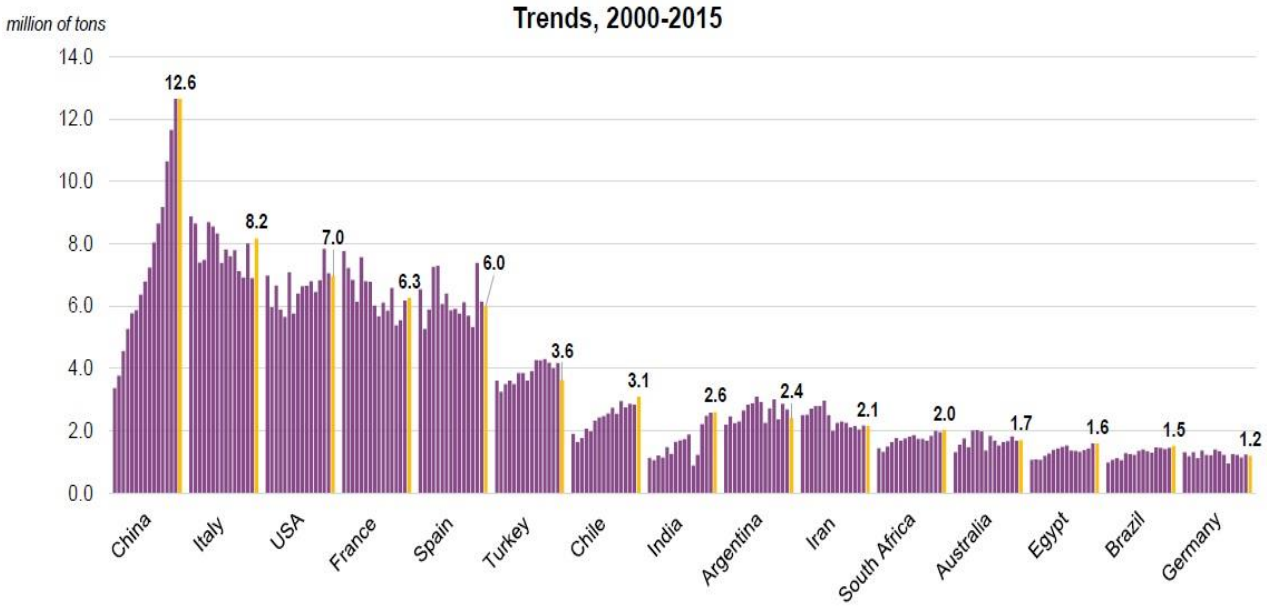


Figure I.1: The trends of grapes production per country from 2000 till 2015 (OIV, 2016)

Like many plants, there is not a single vine variety, but thousands. More than 5000 varieties are listed, and today about 250 of these are cultivated commercially. The varieties are distinguished by their different shapes of leaf, berries and colors and have different aroma and taste profiles. The two most cultivated grape species are: *Vitis vinifera* (From Europe, and from which are derived all major varieties for wine and table grapes); *Vitis labrusca* (From North America, used mainly as table grapes, and relatively few for wines). The ripening of grape is accompanied by loss of fruit firmness, accumulation of sugars, reduced acidity, color change and the synthesis of

aromatic compounds. The skin of grapes has a complex structure of polysaccharides, proteins, lipids, aromatic and phenolic compounds. The grape is a major source of polyphenols, which are a family of organic molecules characterized, as its name indicates by the presence of several phenol groups. Figure I.2 shows the distribution of different classes of polyphenols in the grape berry.

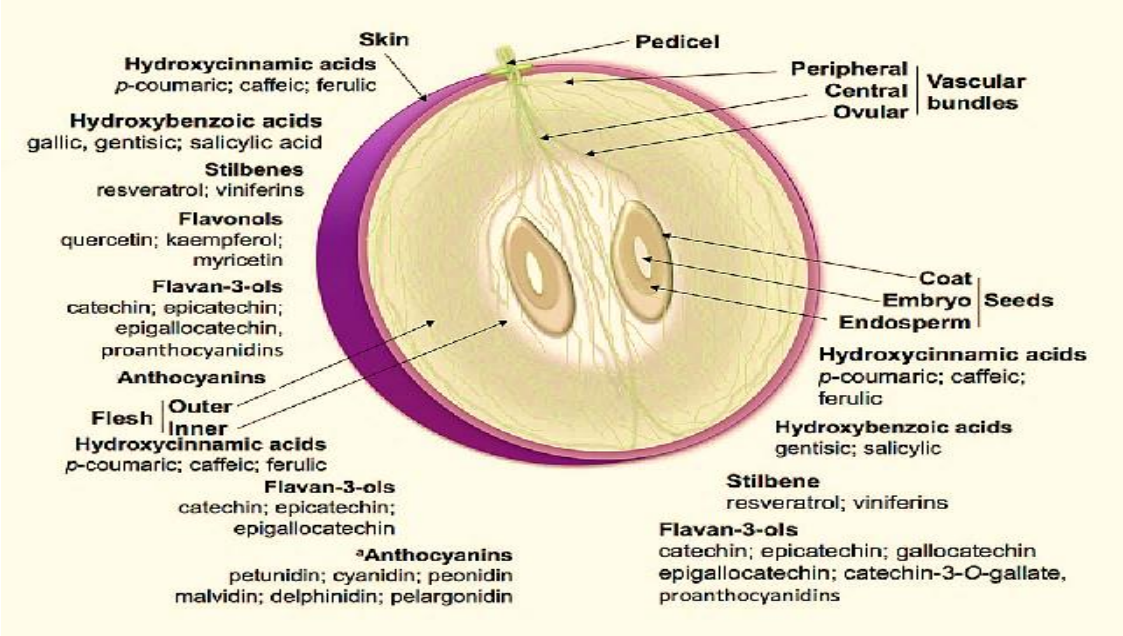


Figure I.2: Schematic structure of a ripe grape berry and pattern phenolics biosynthesis distribution between several organs and tissues (indicated by arrows). [a]Anthocyanins are synthetized also in the inner flesh of the teinturier varieties (Conde et al., 2007)

I.2. Phenolic compounds

Phenolic compounds play a major role in enology. These compounds are the products of plant secondary metabolites responsible for all the differences between red and white wines, especially the color and flavor of red wines. They have interesting, healthful properties, responsible for the „French paradox" which is relatively low rate of coronary heart disease (CHD) in France despite a high dietary intake of cholesterol and saturated fat (Renaud and de Lorgeril 1992). In fact, the role of natural antioxidants attracting more and more interest in the prevention and treatment of cancer, cardiovascular, inflammatory and neurodegenerative diseases (to be discussed in detail in

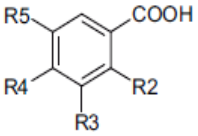
the second part of this chapter). From chemical point of view, the phenolic compounds are characterized by the presence of at least one phenol groups. Two classes are distinguished: Non-flavonoid and flavonoid compounds (Ribéreau-Gayon et al., 2006)

I.2.1. NON-FLAVONOID PHENOLICS

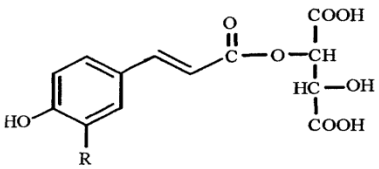
Non-flavonoids cover C6-C3 hydroxycinnamates acids, C6-C1 hydroxybenzoic acids and C6-C3-C6 stilbenes, *trans*-resveratrol, *cis*-resvertarol, and *trans*-resveratrol glucoside (piceid) (Figure I.3)

I.2.1.1. Phenolic Acids

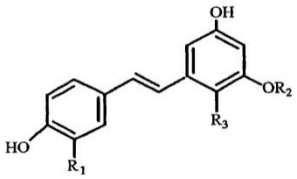
In grapes, phenolic acids are frequently divided in two main groups: hydroxycinnamic and hydroxybenzoic acids. Hydroxycinnamic acids characterized by a C6-C3 skeleton are mainly found as tartaric esters of caffeic, coumaric and ferulic acid in the grape skin and pulp cells (Ribéreau-Gayon, 1965). They are responsible for the phenomenon of browning of wines caused by oxidation. The three basic tartaric structures are: caftaric, coutaric and fertaric esters that differ by the substituents on the aromatic ring (Figure I.3). Caftaric acid is predominant in grapes with an average of 170 mg/kg, 20 mg/kg for coutaric acid and 5 mg/kg for fertaric acid (Singleton et al., 1986). These relative proportions are maintained in the wine. They are mainly present in *trans* isomers, but also exist in *cis* forms (Chira et al., 2008). Hydroxybenzoic acids are characterized by a C6-C1 skeleton, consisting of a benzene ring connected to an aliphatic carbon chain. The most common derivates are vanillic, syringic, gentisic and gallic acid. Grapes mainly contain gallic acid in the pulp (Figure I.3), found in their free and glycoside form. The values range between 100 and 230 mg/kg (Chira et al., 2008).

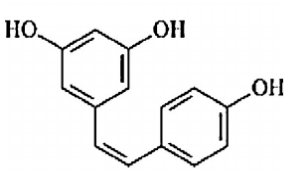


Hydroxybenzoic acids	R2	R3	R4	R5
Vanillic acid	H	OCH_3	OH	H
Syingic acid	H	OCH_3	OH	OCH_3
Gentisic acid	OH	H	H	OH
Gallic acid	H	OH	OH	OH



Esters hydroxycinnamiques	R
trans-caffeoyl tartric acid (caftaric)	OH
trans-p-coumaroyl tartric acid (coutaric)	H
trans-feruloyl tartric acid (fertaric)	OCH_3





Stilbenes	R1	R2	R3
Trans-resveratrol	H	H	H
Piceid	H	glc	H

Trans-resveratrol *Cis*-resveratrol

Figure I.3: Main non-flavonoid compounds found in *Vitis vinifera* grape varieties

I.2.1.2. Stilbenes

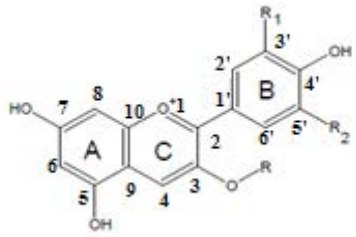
Stilbenes are another minor class of phenolic compounds which have a C6-C2-C6 structure; two benzene rings are linked by a methylene bridge, forming a conjugated system. The principal stilbene in grapes, resveratrol, is produced by vines in response to *Botrytis* infection and other fungal attacks. The actual anti-fungal compounds are the oligomers of resveratrol called the viniferins. Several forms of resveratrol exist including the *cis* and *trans* isomers as well as the glucosides of both isomers. All are found in wine, but in grapes *cis*-resveratrol is absent. The most abundant in grapes are *trans*-resveratrol and its glycosylated derivative: the piceid (Jeandet et al., 1991; Waterhouse and Lamuela-Raventos, 1994) (Figure I.3). Light causes the *cis*/*trans* isomerization. Resveratrol derivatives are found only in the skin of the grape, so much more is found in red wine. So for example, botrytis berries contain higher level of resveratrol (Borie et al., 2004). The total levels of all forms average about 7 mg/l for red, 2 mg/l for rosés and 0.5 mg/l for white wines (Andrew, 2002). The interest in the health effects of resveratrol has generated more than 3300 research papers on resveratrol (Scopus, 2016).

I.2.2. FLAVONOIDS COMPOUNDS

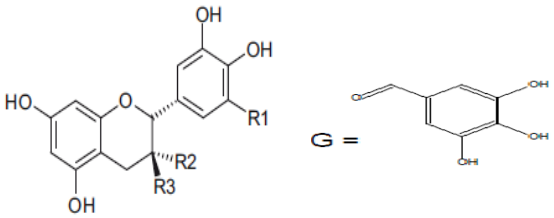
Flavonoids are characterized by a basic structure of 15 carbon atoms including 2 aromatic rings bound through a 3 carbon chain (Figure I.4). These are the most abundant of all the phenolic compounds. They are plant secondary metabolites which are involved in the process of defense against UV, pigmentation and certain disease resistance (Chira et al., 2008). Differences in the oxidation state and substitution on ring C define the different classes of flavonoids. The major classes of grape flavonoids are the anthocyanins, flavanols, flavonols and flavanones.

I.2.2.1. Anthocyanins

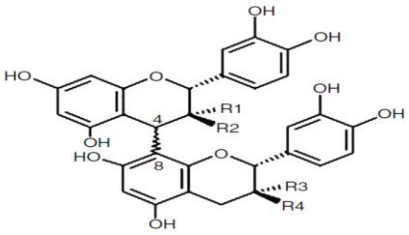
Anthocyanins provide the red and blue colors found in the skins of red or black grapes (Amrani Joutei, 1993). A number of physical conditions also affect anthocyanin stability, such as temperature, light, oxygen, metals, etc. Anthocyanins are located mainly in the skin and, more unusually, in the flesh of „teinturier" grape varieties. They are also present in large quantities in the leaves, mainly at the end of the growing season. They are characterized by a core glycosylated flavylium in position C-3 that combines two benzene rings A and B (Figure I.4). The variation of degree of methoxylation and hydroxylation of the B ring leads to the five aglycones found in *Vitis vinifera* varieties: Delphinidin, Petunidin, Malvidin, Cyanidin and Peonidin (Figure I.4). Unlike other hybrids (*Vitis riparia* and *Vitis rupestris*), which occur as 3, 5-diglucosides, *Vitis vinifera* contains only traces and is characterized by the predominant presence of malvidin 3-O-glucosides whose content varies between 90% (Grenache) and 50% (Sangiovese) (Chira et al., 2008). Anthocyanins can be divided into subclasses depending on the pattern of substitutions of the glucose C ring. The glucose may be acylated at the 6 position by acetic acid, para-coumaric acid or caffeic acid. Anthocyanins are also capable of forming conjugates with the hydroxycinnamic acids and organic acids (malic acid and acetic acid). For the majority of grape varieties, the most abundant individual anthocyanins are malvidin-3-O-glucoside while cyanidin-3-O-glucoside is the lowest abundant form (Nicoletti et al., 2008). The content and composition of anthocyanins in grapes varies with species and variety (Mazza and Miniati, 1993).



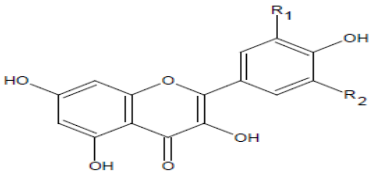
Anthocyanidins	R=H	R1	R2
Delphinidin		OH	OH
Cyanidin		OH	H
Petunidin		OCH_3	OH
Peonidin		OCH_3	H
Malvidin		OCH_3	OCH_3



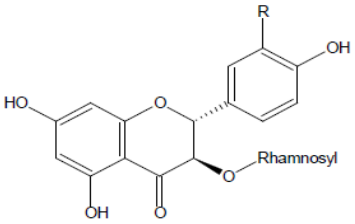
Flavanols monomers	R1	R2	R3
Catechin	H	H	OH
Epicatechin	H	OH	H
Epigallocatechin	OH	OH	H
Epicatechin gallate	H	OG	OG



Flavanols dimers (C6-C8)	R1	R2	R3	R4
Procyanidin B1	OH	H	H	OH
Procyanidin B2	OH	H	OH	H
Procyanidin B3	H	OH	H	OH
Procyanidin B4	H	OH	OH	H



Flavonols	R1	R2
Kaempferol	H	H
Quercetin	OH	H
Myricetin	OH	OH
Isorhamnetin	OCH_3	H
Syringetin	OCH_3	OCH_3
laricitrin	OH	OCH_3

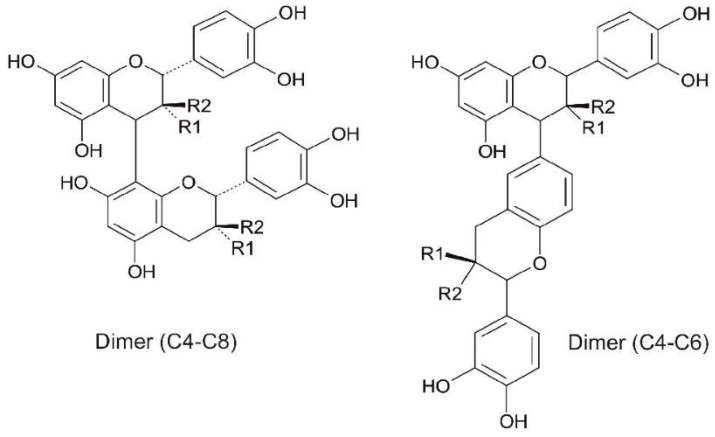


Flavanones	R
Engeletin	H
Astilbin	OH

Figure I.4: Main flavonoid compounds found in *Vitis Vinifera* grape

I.2.2.2. Flavanols

Flavanols are the most abundant class of phenolics in the grape berry; they play an important role on the organoleptic properties of wines, in particular, astringency. They include monomers and condensed tannins (proanthocyanidins), common name for oligomers and polymers of flavan-3-ols (Figure I.4). The monomers are (+)-catechin, (-)-epicatechin, (+)-gallocatechin, (-)-epigallocatechin and epicatechin-3-O-gallate (Escribano-Bailón et al., 1995; Souquet et al., 1996). There are also dimeric, trimeric, oligomeric, and condensed procyanidins (Figure I.5). Dimeric procyanidins are dimers resulting from the condensation of two units of flavan-3-ols linked by a C4-C8 (B1 to B4) or C4-C6 (B5 to B6) bond. Trimeric procyanidins are trimers with two interflavan bonds while oligomeric procyanidins are polymers from three to ten flavanol units linked by C4-C8 or C4-C6 bonds. Condensed procyanidins have more than ten flavan units (Ribéreau-Gayon et al., 2006).



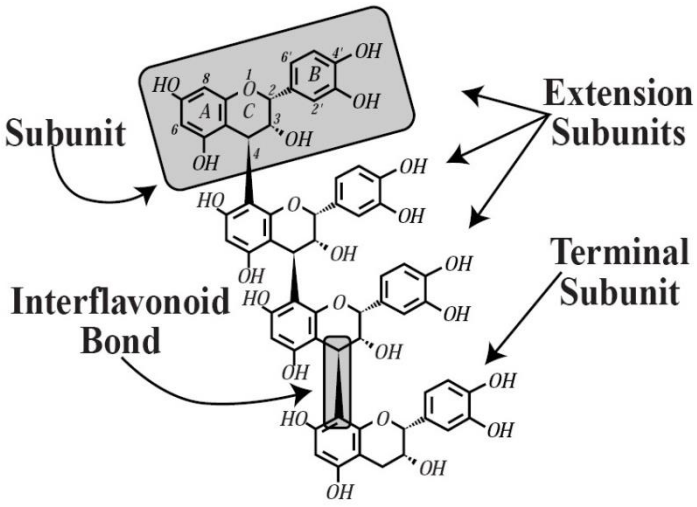


Figure I.5: Chemical Structure of Flavanols dimers and polymers

Skins, seeds and stems are the area of concentration of flavanols especially proanthocyanidins (Spranger et al., 1998; Sun et al., 1999), which are oligomers and polymers of flavan-3-ols that have the property of releasing anthocyanidins in hot and acidic medium, by cleavage of the inter-monomeric bonds from the higher units (Bate-Smith, 1954). There are two types of proanthocyanidins found in grapes according to the nature of the anthocyanidins released: procyanidins (polymers of catechin and epicatechin), which release cyanidin and prodelphinidins (polymers of gallocatechin and epigallocatechin) which release delphinidin.

In the grape berries, tannins are located in the external and internal envelopes of seeds and in the skin cells (Souquet et al., 1996; Mane et al., 2007). The distribution of the flavanols in grape berries is not the same in all varieties, and in fact has a wide range of differences comparing seed and skin tannins. The trihydroxylated forms monomeric forms of flavans-3-ols (gallocatechins) have been identified in grapes under their polymeric forms in both the skin and pulp (Souquet et al., 1996; Mane et al., 2007). Grape seed tannins consist of procyanidins partially galloyles, while skins and stems contain procyanidins and prodelphinidins units (Souquet et al., 1996; Souquet et al., 2000). Procyanidin B1 has been reported to be the main oligomer in skins (Escribano-Bailón et al., 1995; Jordão et al., 2001a), while procyanidin B2 is the most abundant in seeds (Bourzeix at al., 1986; Ricardo-da-Silva et al., 1991).

Besides the nature of the constituent units, the tannins are differentiated by the number of units, called degree of polymerization, as well as the type and position of inter-monomeric bond. The

B-type proanthocyanidins are characterized by an inter-monomeric bond between carbon 4 (C4) of the upper unit and carbon 6 (C6) or the carbon eight (C8) of the lower unit, *trans* configuration with respect to the hydroxyl of carbon 3 (C3). The A-type Proanthocyanidins contained additional ether linkage between the C2 carbon of the upper unit, and carbons 5 or 7 of the terminal unit (Vivas and Glories, 1996). It should be noted that the existence of A-type proanthocyanidins is not confirmed in grapes but only assumed from chromatographic characteristics (Glories et al., 1996; Salagoity Augustus and Bertrand, 1984). Seed tannins consist of procyanidins partially galloyles. Their mean degree of polymerization (mDP) of seed tannins (mDP = 10) is much lower than those of skins; which also contain prodelphinidins, and whose mean degree of polymerization (mPD) is around 30 units (Prieur et al., 1994; Souquet et al., 1996). In seeds and skins, the polymeric tannin fractions are present in a greater proportion than the monomeric or dimeric tannins (Cheynier et al., 1997) depending on the grape variety. In addition, polymeric tannins represent 77-85% of total flavanols in seeds and 91-99% of total flavanols in skins (Cosme et al., 2009). Recently, the presence of tannins of higher degree of polymerization whose characteristics are similar to those of skin tannin was demonstrated within the pulp (Souquet et al., 2006).

I.2.2.3. Flavonols

Flavonols are yellow pigments found in grape skin of both red and white grapes (price et al., 1995). They are characterized by the existence of a double bond between C2 and C3, and a hydroxyl group in C3. This class of compounds is found in a glycoside form but there also significant amounts of glucuronides. Four glycosylated flavonols derivatives from four aglycones (kaempferol, quercetin, myricetin and isorhamnetin, Figure I.4) are mainly present in grapes. Derivates of syringetin and laricitrin have recently been in evidence in red varieties (Mattivi et al., 2006). The average levels of flavonols in grapes are near 50 mg/kg but may vary between 10 and 285 mg/kg (Ritchey and Waterhouse, 1999). Kaempferol and quercetin flavonols are present in both red and white grapes, whereas, myricetin and isorhamnetin occur merely in red grapes (Mattivi et al., 2006; Castillo-Muñoz et al., 2007). A study on Pinot noir has shown that sunlight on the berry skin strongly enhances the levels of the flavonols. Since flavonols absorb UV light strongly at 360nm, and they appear mostly in the outermost layer of cells in the berry, it appears that the plant produces these compounds as a natural sunscreen (Andrew, 2002).

I.2.2.4. Flavanones

This family of compounds was identified in the skins of white grapes, characterized by the presence of a chiral center on the carbon 2. Astilbin and engeletin (3-rhamnosides of dihydroquercetin and dihydrokampférol) are the representative of this family (Figure I.4). These molecules have also been observed in stems (Souquet et al., 1998). In grapes flavanones are present at concentrations of a few mg/kg (Chira et al., 2008).

I.3. Wine phenolic compositions

The comparison of the phenolic composition of grapes and wine shows that alongside of molecules that comes directly from the berries, other polyphenols appear in the wine. During vinification and aging, polyphenolic compounds are involved in various types of reactions, giving rise to a multiplicity of new structures.

I.3.1. ANTHOCYANINS

The anthocyanidins (aglycons form, Figure I.4) are the basic structure of the anthocyanins. When the anthocyanidins are found in their glucoside form (bonded to a sugar moiety) they are known as anthocyanins. The anthocyanins identified in wines from *Vitis vinifera* are the 3-O-monoglucosides and the 3-O-acylated monoglucosides of five important anthocyanidins – cyanidin, delphinidin, petunidin and malvidin that are differenciated by the number and position of hydroxyl and methoxyl groups located in the B-ring of the molecule. Acylation occurs at the C-6 position of the glucose molecule by esterification with acetic, lactic, p-coumaric and caffeic acids (Mazza and Miniati, 1993; Bakowska-Barczak, A., 2005).

The wine anthocyanin composition depends on the original grape profile but also on the extraction and winemaking techniques employed. Anthocyanin concentrations are in the order of 20-500 mg/l in red wine (Flanzy, 1998). Their concentration reaches a maximum in a few days of fermentation and then decreases as a consequence of their adsorption on yeast cell walls, precipitation in the form of colloidal material together with tartaric salts, elimination during filtration and fining (Castillo-Sánchez et al., 2006; Moreno Arribas et al., 2008), as well as, their involvement in many chemical reactions (Ribéreau-Gayon 1982; Somers 1971; Cheynier et al., 1997a; Mayen et al., 1995; Romero-Cascales et al., 2005). These are unstable pigments but their reactivity leads to many pigments which contribute to the color stability of red wines.

Anthocyanins can be found in different chemical forms which depend on the pH of the solution (Figure I.6). At pH 1, the flavylium cation (red colour) is the predominant species of which it is subjected to deprotonation and hydratatation reactions when pH increases (Brouillard et al., 1977). At pH values between 3 and 4, the hemiketal (AOH) species are predominant. At pH values between 5 and 6 only two colourless species can be observed, which are a carbinol pseudobase and a chalcone, respectively. At pH values higher than 7, the anthocyanins are degraded depending on their substituent groups. At wine pH, four structural forms of the anthocyanins coexist: flavylium cation, anhydrous quinoidal base, colourless carbinol base and the pale yellow chalcone. Anthocyanins are frequently represented as their red flavylium cation, but in aqueous media this form suffers rapid proton transfer reactions, leading to blue quinonoidal bases. By the other hand, the hydration generates colorless hemiketals in equilibrium with chalcone structures.

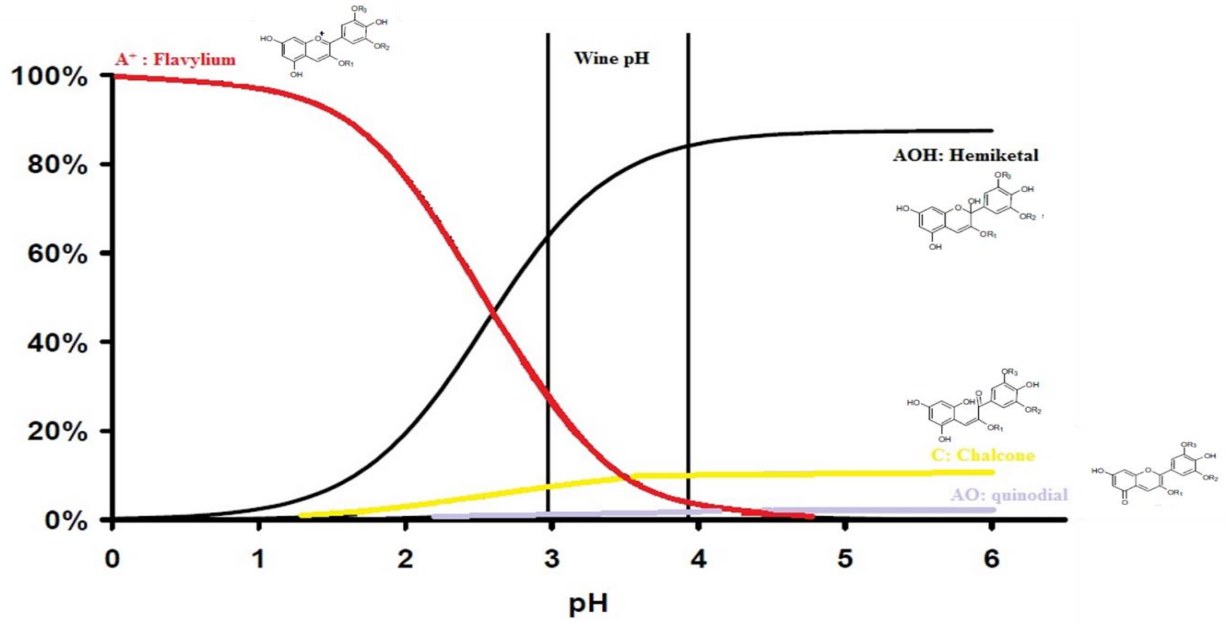


Figure I.6: Anthocyanins chemical forms depending on wine pH (adapted from Brouillard and Dubois, 1977)

I.3.1.1. Reactions and interactions of anthocyanins

The anthocyanins are structurally dependent on the conditions and composition of the media where they are dissolved. In wine, anthocyanins can undergo interactions among them and with other compounds that influence their structural equilibria and modify their color.

I.3.1.1.1 Nucleophilic addition reaction

The A-ring of anthocyanin is nucleophilic whereas the C-ring has a cationic charge and reacts as an electrophile. The addition of bisulfite to the anthocyanin illustrates the best example of a nucleophilic addition reaction onto the flavylium cation. This addition is known as anthocyanin bleaching because the addition of sodium metabisulfite on the carbon 4 of anthocyanins results into the decolorization of anthocyanins (Timberlake and Bridle, 1967; Berke et al., 1998). This reaction is reversible (because of the high value of the oxygenation of the core which is characterized by dissociation constant) and the red flavylium form may be regenerated by acidification or addition of acetaldehyde which combines bisulfite.

I.3.1.1.2. Condensation reactions

In this part, the nucleophilic form of anthocyanins is involved into condensation reactions. Anthocyanins in its hemiketal form can undergo condensation reaction with electrophilic o-quinones (species generated by enzymatic oxidation of caftaric and cutaric acid) resulting into colorless adducts. It is suggested that the anthocyanin is linked to the quinone by its C_6 or C_8 position.

I.3.1.1.3. Self-association of anthocyanins

At high concentrations, the colored form of anthocyanins is associated together to form the non-covalent dimers vertical stack. The self-association is promoted by the hydrophilic interactions between glucose components and by the hydrophobic repulsion between the aromatic ring and water. This phenomenon leads to an intensification of color and a deviation of Beer-Lambert law (Asen, 1972; Goto et al., 1991; Hoshino, 1991). Covalent dimers like $A\text{-}A^+$ can also be formed (Salas, 2005).

The self-association can be recognized as a special form of a copigmentation. It can also influence the apparent hydration constant of the anthocyanins and subsequently modify the color of red wines (He et al., 2012). The presence of ethanol in wines limits the self-association since it can weaken the hydrophobic interactions.

I.3.1.1.4. Copigmentation reactions

The phenomenon of copigmentation is defined as a solution phenomenon in which pigments (anthocyanins in the case of wine) and other non-colored organic components form molecular associations or complexes (Boulton, 2001). It can divided into 2 classes: the intramolecular copigmentation and the intermolecular copigmentation.

The intramolecular copigmentation corresponds to two parts of the same molecule, on which one plays the role of copigment and the other being the chromophore (Brouillard et al., 1993; Dangles et al., 1993; Figueiredo et al., 1996; Goto et al., 1991). For example, in coumaroylated and caffeoylated anthocyanins, the aromatic ring of the acylated part of glucose substitute may cause a stabilization of anthocyanin portion in the form of flavylium.

The intermolecular copigmentation is the result of the vertical stacking between the planar portion of copigment rich in π- electrons and the colored forms of anthocyanins (Brouillard et al., 1989; Cai et al., 1990; Dangles and Brouillard, 1992a; Dangles and Brouillard, 1992b). The colored forms (A^+, AO) have planar structures with a strong delocalization of π-electrons, allowing $\pi - \pi$ stacking with the copigment. The formation of the $\pi - \pi$ complex which causes changes in the spectral properties of the molecules in the flavylium ion, increasing the absorption intensity (hyperchromic effect) and its wavelength (bathochromic shift); and the stabilisation of the flavylium form by the π complex displaces the equilibrium in such way that the red colour increases. The co-pigmentation effect is evident under weakly acid conditions (pH 4–6) where anthocyanins exist in its colourless forms. Recently, it has been proposed that this phenomenon induces the reactions between anthocyanins and tannins in wines (Rein and Heinonen, 2004). Both form of copigmentation cause the pigments to exhibit far greater color than would be expected from their concentration.

I.3.1.1.5. Cycloaddition reactions

Free anthocyanins can undergo cycloaddition reactions or direct reaction with some constituents of the wine; including metabolites of various yeasts (e.g. pyruvic acids, acetaldehyde and vinylephenol) giving rise to pyranoanthocyanin pigments (Figure I.7). Pyranoanthocyanins are cycloaddition products which have an additional pyran ring between the C4 position in the C ring and the hydroxyl group on the C_5 position in the A ring of the anthocyanin molecule. The constitute one of the most important anthocyanin-derived pigments in red wine.

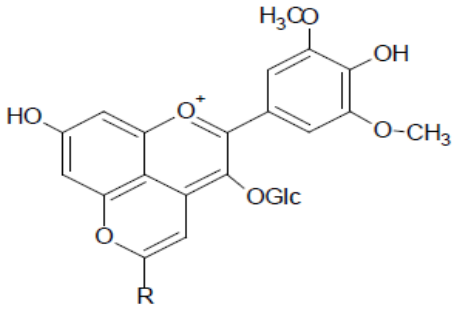


Figure I.7: Structure of pyranomalvidin-3-O-glucoside detected in wine or model solution: R = H, pyranomalvidin-3-O-glucoside; R= COOH, carboxy-pyranomalvidin-3-O-glucoside; R= phénol, 4, hydroxyphenyl-pyranomalvidin-3-O-glucoside; R= monomer or dimer of flavanol, flavanyl-pyranomalvidin-3-O-glucoside

Figure I.8 resumes the different reactions of cycloaddition of free anthocyanin in red wines. Free anthocyanins can react with both hydroxycinnamic acids and 4-vinylphenols leading to the formation of pyranoanthocyanins. The adduct of malvidin-3-glucoside with pyruvic acid is known as vitisin A. The result from the condensation between anthocyanins and acetaldehyde is known as vitisin-B. Portisin is obtained from the reaction between malvidin-3-glucoside–pyruvic acid derivative and (+)-catechin in the presence of acetaldehyde

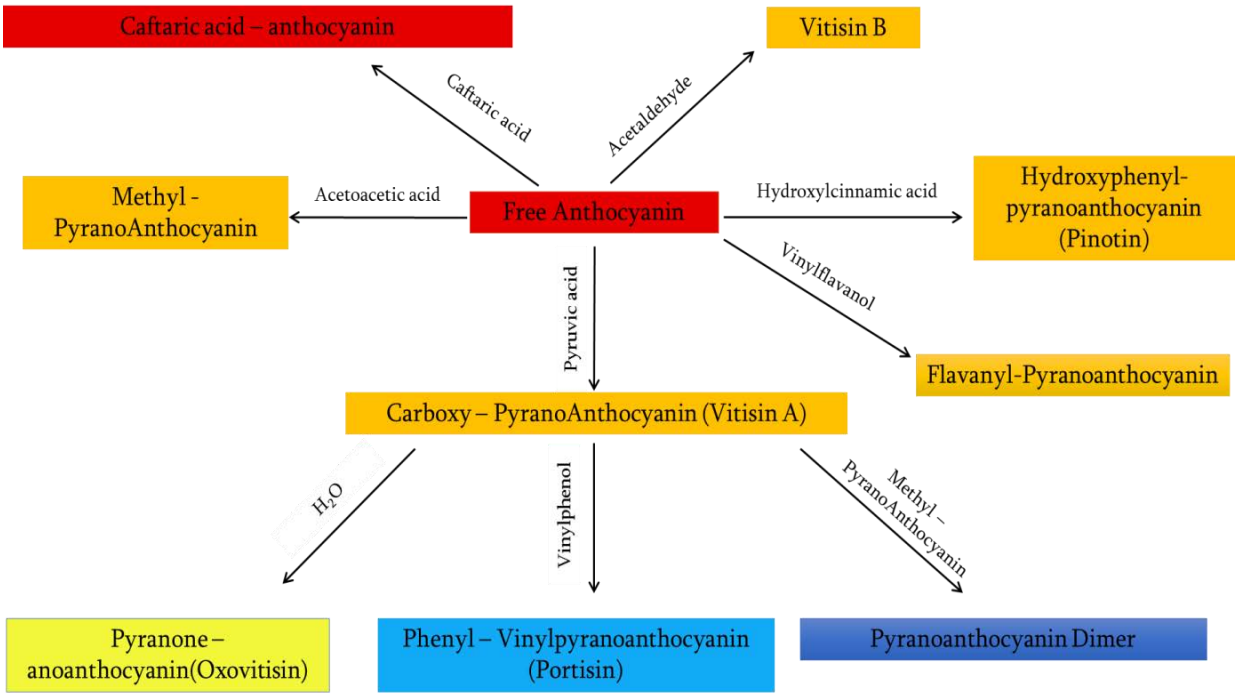


Figure I.8: cycloaddition reaction of free anthocyanins in red wines

The specificity of this reaction lays in the yellow – orange hue of the products formed as well as their remarkable stability, especially toward the changes of pH and the action of sulfites. The UV-Vis spectra of these compounds present a maximum absorbance shifted comparatively to that of the anthocyanins (λ_{max} = 503 nm and 511 nm for pyranoanthocyanidins-flavanol monomers and pyranoanthocyanidins-flavanol dimers respectively, compared to that of anthocyanins (λ_{max} = 529 nm)) (de Freitas and Mateus, 2006). In products that are rich in anthocyanins and possible reaction partners as wine, the formation of pyranoanthocyanins is likely to proceed with increasing storage time.

I.3.2. FLAVANOLS

The concentration of flavanols in red wine varies according to grape variety and, to even greater extent winemaking methods. Values are between 1 and 4 g/l (Ribéreau-Gayon et al., 2006). During the vinification, the extraction of proanthocyanidins is slower than anthocyanins. The proanthocyanidins of skin diffuse more rapidly than those from seeds because of their location and the higher solubility of proanthocyanidins compared to procyanidins galloyles. The extraction of proanthocyanidins in seeds starts when the content in alcohol increases (Labarbe,

2000; Canals et al., 2005; Cheynier et al., 1997a). Tannins are reactive compounds which can react with anthocyanins or other tannins to form derivatives of tannins-tannins or anthocyanins-tannins in wine. This reactivity is due to the chemical structure of these compounds containing a nucleophilic ring A, an oxidized ring B and an electrophilic ring C (cationic form only, Figure I.9). They also have particular physico-chemical properties that combine to form aggregates and interact with proteins and polysaccharides.

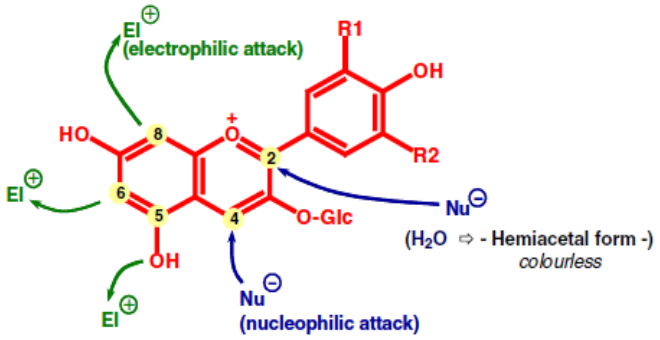


Figure I.9: Schematic representation of the main reactive position of anthocyanin structures

The first reaction is deccribed by a direct condensation reaction between anthocyanin-tannin leading to an A-T adduct (Figure I.10). In this reaction, anthocyanins act as cations (A^+) on the negative nodes (6 or 8) of the procyanidins (P), forming a colorless flavene (A-P). The presence of oxygen or an oxidizing medium is necessary for the flavene to recover its color. The forms are in balance: A^+-P and AO-P (Figure I.10) (Hrazdina and Borzell, 1971; Liao et al., 1992; Salas, 2005; Santos-Buelga et al., 1995; Somers, 1971; Timberlake and Bridle, 1976).

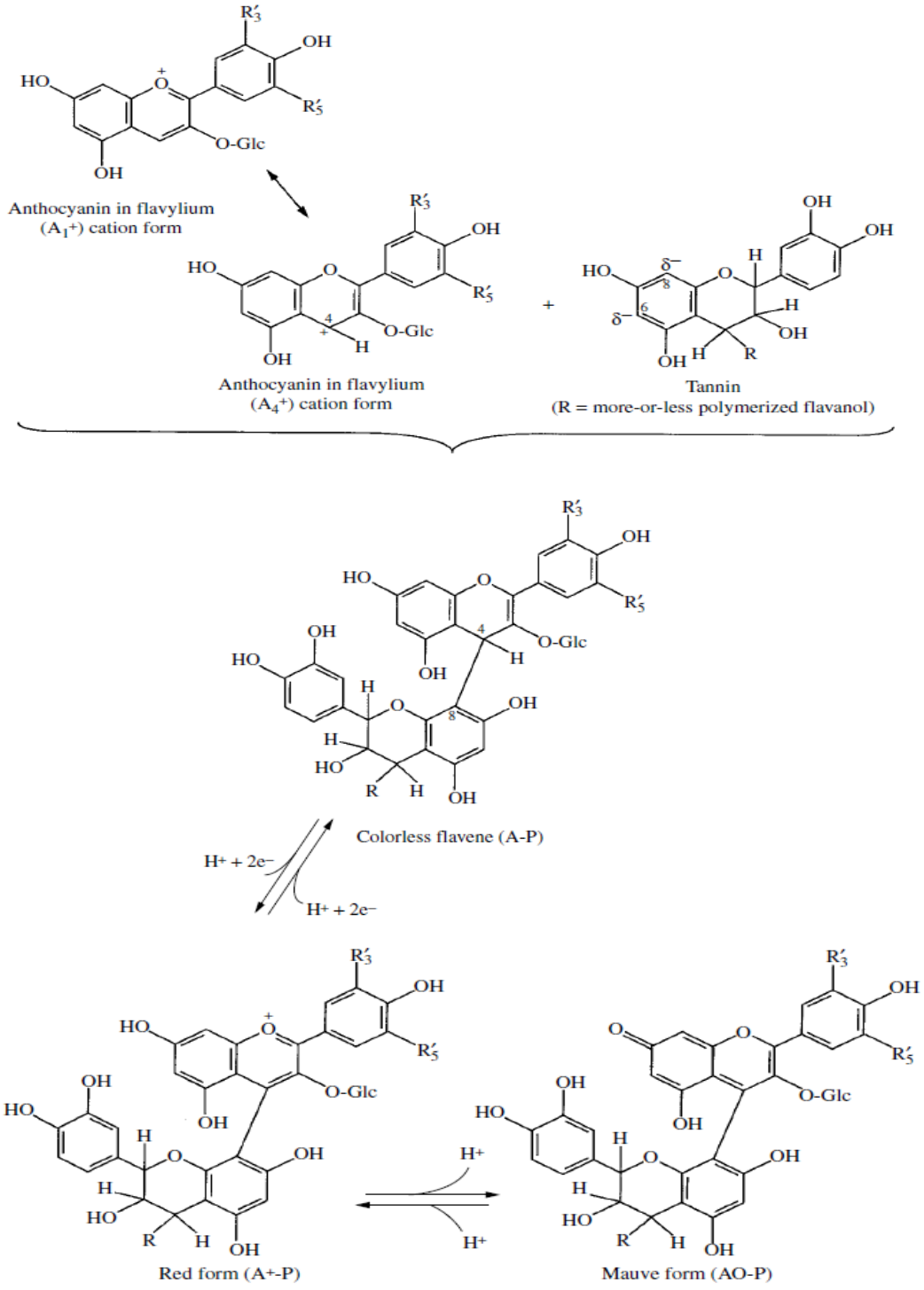


Figure I.10: Direct A-T type condensation of anthocyanins and tannins (Galvin, 1993)

The second reaction is characterized by a direct condensation between tannin-anthocyanin and tannin-tannin leading to T-A and T-T adduct respectively. One of the characteristics of

procyanidins is that they form a carbocation after protonation of the molecule, and react with nucleophilic sites, such as nodes 6 and 8 of anthocyanin molecules as carbinol bases (neutal) (Somers, 1971) (Figure I.11). The complex thus formed (T-AOH) is colorless and turn a reddish-orange color on dehydration (T-A) (Salas et al., 2003). Their presence in wines has been demonstrated in the forms T-A- A^+ (Alcalde - Eon et al., 2007). Carbocations released by breaking of the interflavanic bonds of tannins can react with another molecule of flavanol to give a new tannin molecule. These mechanisms of breakage and recombination can lead to either an increase in the average degree of polymerization of tannins, or a decrease of the latter if the medium contains an excess of monomer units (Haslam, 1980; Vidal et al., 2002).

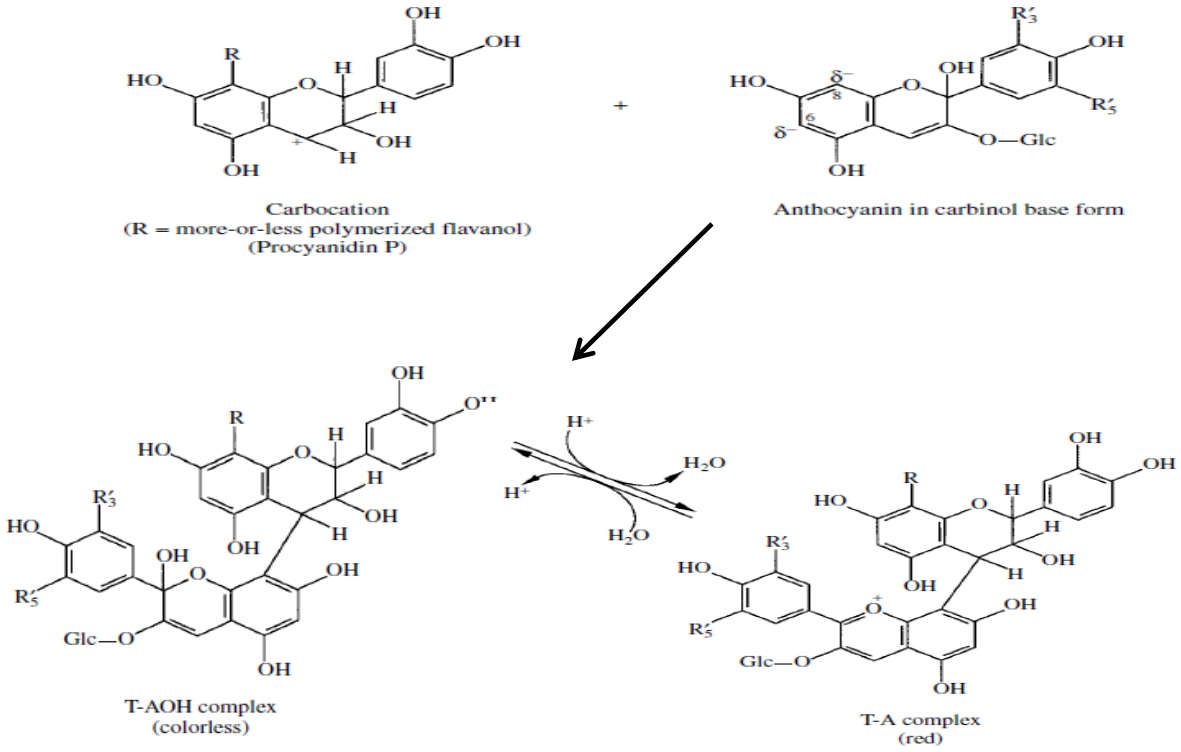


Figure I.11: Direct T-A type condensation of procyanidins and anthocyanins (Galvin, 1993)

The third reaction is represented as indirect reaction, occurs involving aldehydes such as acetaldehyde, which is formed by decarboxylation of pyruvic acid (Liu and Pilone, 2000) or gradually during wine aging resulting from the ethanol oxidation (Wildenradt and Singleton, 1974). Their formation mechanism starts with the protonation of the aldehyde, followed by addition of the resulting carbocation to a nucleophilic position of the flavanol unit (C6 or C8 of

the phloroglucinol ring): the dehydration of the resulting protoned adduct yields a new carbocation, which suffers a nucleophilic attack by the anthocyanin (figure I.12). The resulting product of this reaction is a flavanol anthocyanin adduct wherein the rings A of the two flavonoids are linked by a methylmethine bridge (CH-CH3), commonly called "Ethyl Bridge"". The adduct flavanol-ethyl-anthocyanin, initially in the form of a hemiacetal, gives the corresponding flavylium cation by deshydration and protonation. The flavanol acetaldehyde intermediate may also react with another molecule of flavanol to form catechol dimers on which the units are connected together by an ethyl bridge CH-CH3. The presence of dimeric and trimeric structures of catechin-ethyl-catechin (Cheynier et al., 1997b; Saucier, 1997), and catechin-ethyl-anthocyanin (Atanasova, 2003; Es-Safi et al.; 1999b) has been demonstrated in model solutions and wines, confirming the formation of these compounds during winemaking. In general, the alkyl interflavonoid linkage induces a bathochromic shift of around 15 nm (540 nm) of malvidin-3-glucoside (525nm) and the pigments solutions acquired a more red-purple colour (de Freitas and Mateus, 2006). Moreover this pigment has a high resistance to discoloration by sulfur dioxide when the pH increases comparatively to Mv that could be explained by a greater protection of the chromophore moiety and namely carbon 2 in the pyranic ring, against the nucleophilic attack by water (Freitas and Mateus, 2006).

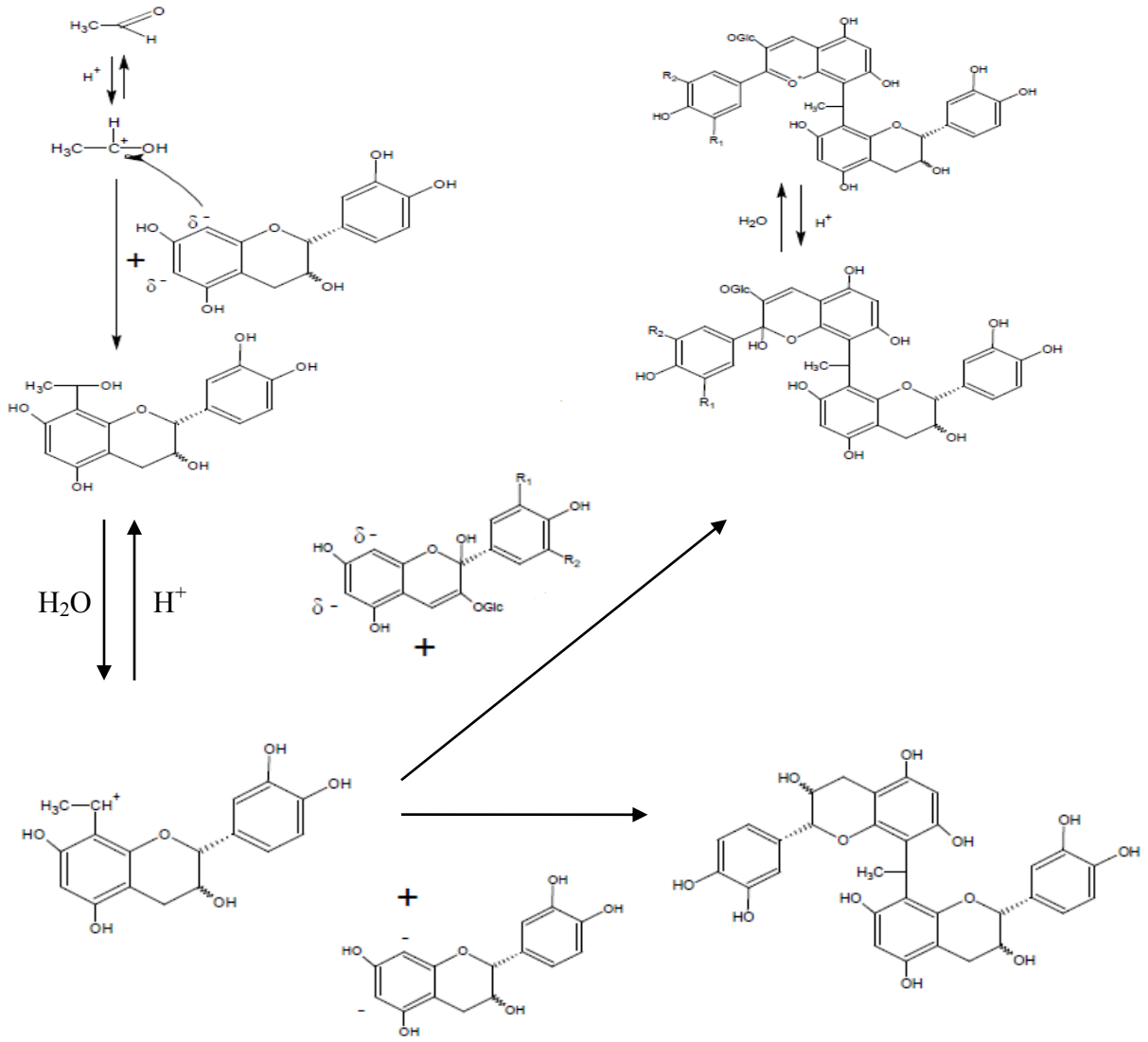


Figure I.12: Mechanism of formation of flavanol-ethyl-flavanol and flavanol-ethyl-anthocyanin adducts by condensation reaction mediated by acetaldehyde

The self-association of flavanols and their aggregation have been demonstrated in the literature (Poncet-Legrand et al., 2003; Pianet et al., 2008). It was demonstrated that hydrophobic interactions are the major driving forces to the flavanols self-association. Flavanols may react also with other wine macromolecules as proteins through hydrophobic effects and hydrogen bonding (Luck et al., 1994), and the interaction polyphenol-protein is modulated by several factors: size, structure and solubility of polyphenols, ethanol concentration, stoichiometric ratio of polyphenols, proteins, pH and composition of the medium. The reaction of some polysaccharides like mannoproteins and arabinogalacturonan proteins with tannins prevent the

agglomeration and precipitation of these latter and limits the precipitation of tannin-proteins complexes.

I.3.3. FLAVONOLS AND FLAVONES

Flavonols (Figure I.13) constitute a group of flavonoids that are closely related in structure to the flavones. Their concentrations in red wine range from 10 to 80 mg/l (Flanzy, 1998). Flavones are represented mainly by kaempferol, quercetin and myricetin. The major flavonols in wine are 3-glycosides and 3-glucuronide of quercetin and myricetin. Flavonols, when they occur in their deglycosylated form, are labile molecules and may be degraded upon exposure to heat, enzymes and oxidative chemical species (Markis et al., 2006). In addition, in wines common winemaking practices, including maceration, fermentation, ageing and storage conditions are responsible for significant changes in flavonols. (Castellari et al., 2000), showed that supplementation with oxygen during storage decreased quercetin levels by more than 50% over a period of 6 months

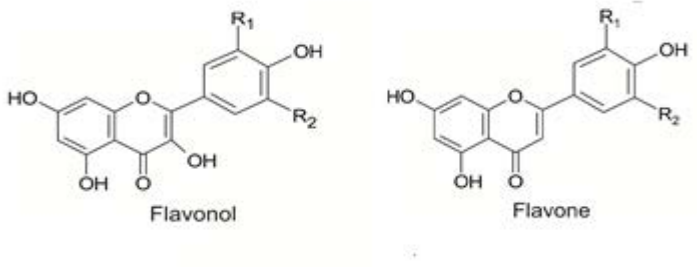


Figure I.13: Chemical structures of flavonol and flavone. R_1 and R_2 could be H, OH or OCH_3

I.3.4. PHENOLIC ACIDS

I.3.4.1. Hydroxybenzoic Acids.

Already described in I.2.1.1. (see page 9). The most common derivatives found in wine are gallic, gentisic, p-hydroxybenzoic, protocatechuic, syringic, salicylic and vanillic acid. Gallic acid is the most abundant hydroxybenzoic acids in wine whose concentration ranging from 2 to 130 mg/l (Flanzy, 1998). It not only originates from the grape itself but is also formed by hydrolysis of hydrolysable and condensed tannins (gallic acid esters of flavan-3-ols). The levels of hydroxybenzoic acids in wine show great variability depending on grape variety and growing conditions. The levels of hydroxybenzoic acids and their derivatives are commonly low in wine, compared to the levels of hydroxycinnamic acids (Ribéreau-Gayon et al., 2006; Kelebek et al.,

2009). Concentrations of hydroxybenzoic and hydroxycinnamic acids are in the order of 100-200 mg/l in red wine (Ribéreau-Gayon et al., 2006).

I.3.4.2. Hydroxycinnamic Acids

Already described in I.2.1.1. (see page 9). In wine, hydroxycinnamic acids are present in low amounts in their free form. The majority of phenolic acids present in wine are the caftaric (7-200 mg/l, Flanzy, 1998) and coutaric acid (2-20 mg/l, Flanzy, 1998). There is also fertaric acid in lower concentration. Hydrolysis of such esters takes place naturally, but may be amplified by the action of esterases. The caftaric, p-coumaric and fertaric esters are then transformed into caffeic (0.3-26 mg/l, Flanzy, 1998), ferulic (0.1 mg/l, Flanzy, 1998) and p-coumaric acids (0.4-15 mg/l, Flanzy, 1998). They are involved in chemical oxidation phenomena that lead to browning of grape juice and wine (Cheynier et al., 1989; Mane et al., 2007). Their influence on the taste of wine seems to be less important (Noble and Shannon, 1987; Verette et al., 1988). However, the degradation of p-coumaric and ferulic acid leads to the formation of volatile phenols (vinyl and ethyl phenol, ethyl-vinyl- guaiacol) responsible of olfactory defects (Chatonnet et al., 1993).

I.3.5. STILBENES

Already described in I.2.1.2. (see page 10). The grapes and wines are considered one of the most important dietary sources of these compounds. In grapes, resveratrol is synthesized almost entirely in the skin and the synthesis peaks just before the grapes reach maturity. The levels of resveratrol peak approximately 24 h after stress exposure, and decline after 42-72 h as result of activation of stilbene oxidase (Stervbo et al., 2007). During winemaking stilbenes are transferred into the must and wine. Concentrations in red wines are in the order of 1-3 mg/l (Ribéreau-Gayon et al., 2006).

I.4. Phenolic composition of wines aging in barrels

The main phenolic compounds extracted from the wood to the wine during barrel ageing are (hydrolysable tannins, phenolic acids and wood aldehydes) (Cano-Lopez et al., 2010).

a. **Hydrolysable tannins:** This term refers to both ellagitannins and gallotannins. Ellagitannins are the major phenolic compounds of oak, that they are involved in several reactions with the other phenolic constituents of wine (Michel et al., 2011). Several types

of monomers of ellagitannis exist, whose vescalgin and castalgin are largely predominant in the fagaceous woody species of Quercus (Fernández de Simón et al., 1999, Figure I.14). Ellagitannins are composed of 15 OH groups per molecule and are more readily oxidized than wine Favonoids to produce hydrogen peroxide. Hydrogen peroxide leads to acetaldehyde production (Wildenradt et al., 1974), molecule that is incorporated into red wine phenolic polymers (tannin-ethanal-anthocyane) (Drinkine et al., 2007).

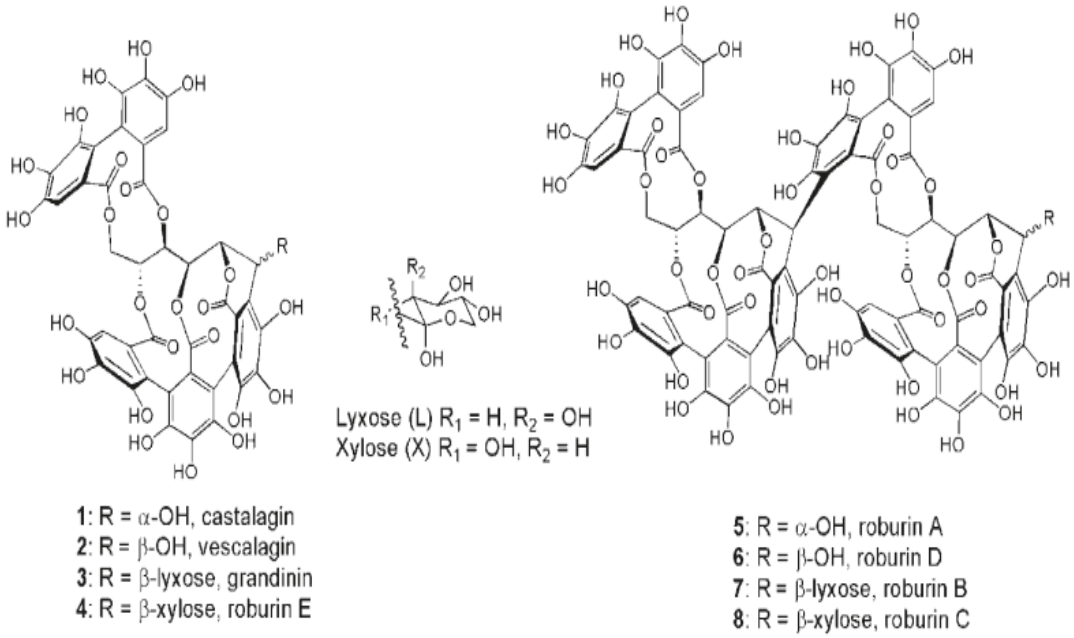


Figure I.14: Structure of main monomeric ellagitannins, vescalagin (2), castalagin (1), as well as the grandinin (3) and roburin A_E (4_8) isolated from Castanea (chestnut) and Quercus (oak) species

b. Great number of complex reactions with other phenolics or with oxygen (Gambutti et al., 2010).

c. **Vanillin:** one of the main aldehydes released from oak wood, leads to the formation of an anthocyanin–catechin purple pigment, by condensation reactions with wine phenolics (Sousa et al., 2007).

I.5. Polyphenols Biological Properties

Both flavonoids and non-flavonoid phenolic compounds have been described as potent antioxidants as they reduce harmful low-density lipoprotein (LDL) cholesterol oxidation, modulate cell signaling pathways, reduce platelet aggregation, inhibit the growth of some tumor types and exhibit anti-inflammatory, antibacterial, antifungal, antiviral, neuroprotective, antiproliferative and antiangiogenic activities (Guilford and Pezzuto, 2011). However, the beneficial effects of moderate wine consumption may be attributed to the overall mix of all it is components and not to a specific action of one.

Polyphenol compounds are widely studied for their antioxidant properties. The increase of the reactive oxygen species and reactive nitrogen species in the body leads to oxidative stress that damage all components of the cell, including proteins, lipids and DNA. Oxidative stress is also associated with chronic diseases, including atherosclerosis, heart failure, cancer, neurological degeneration and aging process. As antioxidants, polyphenols may protect cell constituents against oxidative damage and, therefore, limit the risk of various degenerative diseases associated to oxidative stress. Many mechanisms have been proposed for polyphenol prevention of oxidative stress. The widely studied mechanism is the radical scavenging. In this mechanism, polyphenols reduce the reactive oxygen species (Figure I.15) and reactive nitrogen species preventing the damages.

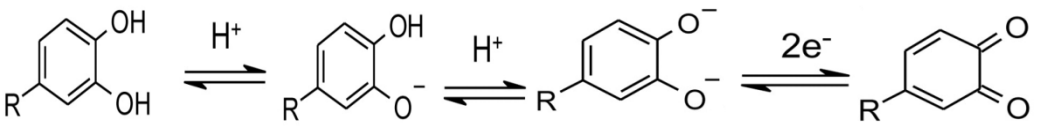


Figure I.15: Polyphenol/quinone redox couples and protonation equilibria (Danilewicz, 2012)

Another mechanism of action to prevent oxidative stress damage concerns the induction of antioxidant enzymes, which act as critically important regulators in cell protection from oxidative stress and chemical-induced damage by controlling the intracellular redox status. Other mechanism of action have been suggested such as chelation of transition metals (cupper and iron) which act as catalysts of oxidative stress, inhibition of reactive oxygen species generating enzymes and modulation of gene expression (ARE/Nrf-2 pathway) (Rodrigo et al., 2011). Red

wine polyphenols has been shown to protect against each of these conditions by increasing plasma antioxidant capacity, suppressing reactive oxygen species generation, increasing serum oxygen radical absorbance capacity, and decreasing oxidative DNA damage (Guilford and Pezzuto 2011).

Research on the beneficial effects of wine polyphenols on human health has received an added impulse with the discovery of the "French paradox" (Renaud and de Lorgeril, 1992). The "French Paradox" is based on epidemiological studies that report the relatively low incidence of cardiovascular disease in the French population despite a relatively high dietary intake of saturated fats. This fact was potentially attributed to the consumption of red wine. Cardiovascular diseases are the leading cause of death worldwide in both men and women. Moderate wine consumption (one or two glasses daily) has been associated with decreased cardiovascular mortality and decreased risk of heart disease (de Gaetano et al., 2003). These benefits have been attributed to increased antioxidant capacities, anti-inflammatory effects, decreased platelet aggregation, improved endothelial function, and increased fibrinolysis. Red wines have exhibited positive effects on biomarkers of atherosclerosis in healthy humans, including a decrease in the LDL/HDL ratio, fibrinogen levels, lipoprotein and clotting factors (Sharpe et al., 1995; Avellone et al., 2006). Endothelial cells play a major role in regulating the balance between the synthesis and interaction of proteins that promote clot formation and fibrinolytic proteins that facilitate fibrinolysis. Short-term ingestion of red wine improved endothelial function in patients with coronary artery disease (Whelan et al., 2004) by promoting endothelium nitric oxide production with vasorelaxing effects, on which this latter was associated with lower blood pressure (Carolo et al., 2007).

Literature shows that moderate wine consumption due to the presence of polyphenols may decrease the risk of several cancers, including colon, basal cell carcinoma, ovarian, and prostate (Bianchini and Vainio 2003). The moderate wine consumption was associated with a decreased risk of esophageal adenocarcinoma (Kubo et al., 2009) and lung cancer (Chao 2007). In vitro and animal studies (Oak et al., 2005) indicate that red wine polyphenols inhibit angiogenesis by reducing the proliferation and migration of endothelial and vascular smooth muscle cells and the expression of proangiogenic factors (Vascular endothelial growth factor [VEGF] and matrix metallopro-teinase-2). Evidence that wine polyphenols contribute to the chemoprotective effects of wine come from studies performed with grape seed proanthocyanidin extraxt (GSPE). GSPE

exhibited toxicity toward human breast, lung, and gastric adenocarcinoma cells, but not normal cells (Bagchi et al., 2002; Katiyar, 2008). Eng et al., 2002 found that red wine polyphenols have an inhibitory activity against aromatase (a cytochrome P 450) involved in breast tumor growth.

In human and animal studies, grape juice and grape extract supported immune function and anti-inflammatory effects, supporting a role for wine polyphenols (Zern et al., 2005; Castilla et al., 2006, 2008). Polyphenol components of wine are capable of protecting against various immune-related disorders by both stimulating the innate and adaptive immune responses as well as reducing inflammation. This effects appears to be associated with the suppression of inflammatory cytokine release (such as nuclear factor-kappa B), induction of anti-inflammatory cytokine release and other protective molecules (interleukins 1α, 6, 10, 12, and interferon-gamma) and the release of nitric oxide (NO) from peripheral blood mononuclear cells.

Epidemiological, clinical, and experimental data supporting positive effects of light-to-moderate wine consumption on lung function, chronic obstructive pulmonary disease progression, the risk of developing lung cancer, acute respiratory distress syndrome and high altitude pulmonary edema (Schafer and Bauersachs 2002; Kamholz, 2006). Proposed mechanisms for pulmonary protection include suppression of endothelin-1 expression, inhibition of inflammatory cytokine release, and antioxidative properties (Culpitt et al., 2003).

Wine polyphenols were exhibited antibiotic activity against *Helicobacter pylori* isolates and protected against associated gastric damage in mice (Daroch et al., 2001; Mahady et al., 2003; Ruggiero et al., 2007; Martini et al., 2009).

Metabolic syndrome is defined by the presence of metabolic risk factors associated with high risk of developing diabetes type II and cardiovascular diseases. These risk factors include abdominal obesity, high plasma triacylglycerols, low plasma HDL, high blood pressure and high fasting plasma glucose. A mechanism that may be important is the ability of wine polyphenols to enhance the function of endothelial NO synthase (eNOS), which may not function properly in metabolic syndrome patients (Leighton et al., 2006; Liu et al., 2008).

Epidemiological and animal studies have demonstrated that moderate red wine intake may reduce the risk of developing neurological disorders, such as dementia, stroke, and Alzheimer‟s disease (Letenneur, 2004; Pinder and Sandler 2004). Oxidative stress resulting in ROS generation is responsible of many forms of cellular deterioration leading to various chronic pathologies like neurodegenerative disorder. The antioxidants effect of polyphenols protects cell

constituents against oxidative damage in the brain that is associated with the process of aging. de la Torre et al., 2006 discovered that some wines contain hydroxytyrosol, a dopamine metabolite and potent antioxidant which can modulate dopamine signaling in the brain. Also, wine polyphenols may regulate the nitric oxide activity at the level of endothelial nitric oxide synthase (eNOS) protein expression in endothelial cells (Wallerath et al., 2003). A preventive approach to reduce tissue injury associated with the risk of cerebral ischema is constituted by eNOS up-regulation by wine polyphenols.

Diabetes type II is characterized by decreased disposal of glucose in peripheral tissues, insulin resistance, over production of glucose by the liver, and defects in pancreatic beta-cells. Long term effects of diabetic patients include an increased risk for cardiovascular disease, blindness, nerve and kidney damage, and limb amputations. Wine polyphenols may affect glycemia through different mechanisms including: the inhibition of glucose adsorption in the gut or it is uptake by peripheral tissues; the inhibition of β-glucosidase, α-amylase and sucrose in rats: the inhibition of gluconeogenesis, adregenic stimulation of glucose intake or the stimulation of insulin by pancreatic β-cells and the protection against beta-cell loss: the modulation of SIRTI gene improving whole-body glucose homeostasis and insulin sensitivity in rats (Marfella et al., 2006; Kar et al., 2009; Zumino, 2009). The antioxidant and the anti-inflammatory properties of wine polyphenols may be responsible for the positive response in type 2 diabetes.

I.5.1. ANTHOCYANINS

Anthocyanins are plant pigments belonging to a subset of flavonoids with a particularly high antioxidant capacity and concomitantly strong health-promoting effects (Bártiková et al., 2013; Yoo et al., 2010). Besides their properties to modulate cognitive and motor function, Anthocyanins may alter specific pathophysiological processes related to various neurodegenerative disorders to improve learning and enhance memory, and to have a role in preventing age-related declines in neural function (Tan et al., 2014). As part of the human diet, they offer protection against cancer, inhibiting the initiation and progression stages of tumor development (Martin et al., 2013). Anthocyanins have been show to inhibit hyperglycemia (type II), improve beta-cell function and protect against beta-cell lost (Zunino, 2009). They also reduce inflammatory inducers of tumor initiation, suppress angiogenesis, and minimize cancer induced DNA damage in animal disease models. Moreover, Anthocyanins also protect against

cardiovascular diseases and age-related degenerative diseases associated with metabolic syndrome (Renaud and de Lorgeril, 1992). Grape juice and wine anthocyanins in synergy with other flavonoids have been cited as responsible for antiplatelet activity in human and dog systems (Shanmuganayam et al., 2002).

I.5.2. FLAVANOLS

Flavanols are present either as monomers ((-)-epicatechin, (+) catechin and gallocatechin gallate), as oligomers and polymers also called condensed tannins or proanthocyanidins. Catechin and proanthocyanidins have proved to be potent antioxidants in different in vitro systems, and in human subjects. It seems that the proanthocyanidin dimer have the most antioxidant effect. These effects confer to the flavanols a cardioprotection action by limiting the oxidative stress factors. However their potential beneficial effect on cardiovascular health is not merely attributed to their antioxidant activities but includes the different mechanisms implicated on cardiovascular conditions or problems, i.e., atherosclerosis, hypertension, platelet aggregation, inflammation, endothelial function, hyperglycemia and hypercholesterolemia (Wang et al., 2002; Jimenez et al., 2008). Wine procyanidins have been shown to be especially active in preventing lipid oxidation of foods while in the digestive tract (Ursini and Sevanian 2002) whereas, wine catechins have strong antimicrobial activity against *Porphyromonas gingivalis and Prevotella intermedia.* A study conducted by Butt and Sultan, 2009 found that in breast cancer cell lines, epicatechin inhibits metastatic cell, hepatocyte growth factor signaling and cell motility; causes cell arrest in S phase; modulates NO signaling, induces Killer caspaces, and inhibits NF-kB signaling. Grape seed proanthocyanidins exhibited toxicity toward human breast, lung and gastric adenocarcinoma cells, but not normal cells (Bagchi et al., 2002; Katiyar, 2008). It protected against tobacco tocixity in oral cells, chemotherapy toxicity in liver cells, and ultraviolet toxicity in skin cells. They exert their anti-cancer effects through the inhibition of the constitutive expression of various NF-kB responsive genes/ proteins such as cyclooxygenase-2, inducible nitric oxide synthase, proliferating cell nuclear antigen and MMP-9 in human epidermoid carcinoma A431 cells (Nandakumar et al., 2008). Human studies have demonstrated that grape seed extract indicating a decreased risk of myocardial infarction by increasing adiponectin levels (Sano et al., 2007; Imhof et al., 2009)

I.5.3. PHENOLIC ACIDS

Phenolic acids represent important fraction of wine phenolics, but their biological effects have been scarcely investigated. The interrelationship between antioxidative capacity and vasodilatory activity, two potentially beneficial biological effects, of phenolic acids from wine were examined. Antioxidative and vasodilatory effects of phenolic acids relate to the number of hydroxyl groups in the aromatic ring (Rice-Evans et al., 1996), degree of compactness and branching of molecules, and three-dimensional distributions of atomic polarisability of the tested molecules (Konstantinova, 1996). Caffeic acid has been shown to have neuroprotective effects against beta-amyloid peptide (Aβ) induced neurotoxicity, against injury induced by 5-S-cysteinyl-dopamine, and by inhibiting peroxynitrite induced neuronal injury (Donggeun et al., 2009; Vanzour et al., 2010). Ferulic acid provides meaningful synergistic protection against oxidative stress in the skin and should protect against photoaging and skin cancer (Lin et al., 2005), hypoglycemic and hypolipidemic effects (Sri Balasubashini et al., 2003; Ohnishi et al., 2004; Jung et al., 2007), hypotensive effects (Suzuki et al., 2002), and anti-inflammatory effects (Yagi and Ohishi, 1979).

I.5.4. FLAVONOLS

Flavonols have been linked to many positive benefits (Krishnaiah et al., 2001, Qin et al., 2011). In this context quercetin is one of the most often studied flavonol ubiquitously present in various vegetables as well as in tea and red wine (Hertog et al., 1993). Passed on the antioxidant properties of quercetin and the association between aging and oxidative stress, Chondrogianni et al., 2010 showed the positive influence of quercetin established on survival, viability, and lifespan of primary human fibroblasts (HFL-1). Quercetin has been recently shown as a potential drug against allergy; that blocks substances involved in allergies and is able to act as an inhibitor of mast cell secretion, causing a decrease in the release of tryptase, MCP-1 and IL-6 and the down-regulation of histidine decarboxylase (HDC) mRNA from few mast cell lines (Shaik et al., 2006). However, there are strong evidences that quercetin as well as related flavonols exert in vitro protective effects on nitric oxide and endothelial function under oxidative stress, endothelium-independent vasodilator and platelet anti-aggregant effects, inhibition of LDL oxidation, reduction of adhesion molecules and other inflammatory markers, prevention of neuronal oxidative and inflammatory damage (Perez-Vizcaino and Duarte, 2010). Quercetin

have been linked to protective effects against several specific cancers, including blood, brain, lung, uterine, prostate and salivary gland cancer by the pro-apoptotic activity in cancer cells; in fact, quercetin is a forthright inhibitor of PI3K, NF-B, and other kinases involved in intracellular signaling (Chirumbolo, 2013). Moreover, In vivo experiments substantiate the anti-inflammatory effect of quercetin which inhibits the production of enzymes usually induced by inflammation (i.e. cyclooxygenase [COX] and lipoxygenase [LOX]) (Kim et al., 1998; Lee et al., 2010). In addition it was reported the antidiabetic effect of quercetin (type 2) which is fulfilled by stimulating glucose uptake through an insulin-independent mechanism involving adenosine monophosphate-activated protein kinase (AMPK) whose activation in skeletal muscle leads to the glucose transporter GLUT4 translocation to the plasma membrane (Eid et al., 2015). Some in vivo studies report (Rao and Vijayakumar, 2008) a protective effect of quercetin against ethanol-induced gastric ulceration as well as against the oesophagitis reflux.

I.5.5. RESVERATROL

Resveratrol is a stilbene naturally occurring phytoalexin released by spermatophytes in response to injury. It is thought to be one of the principal agents in the health-promoting effects of red wine (Baur and Sinclair, 2006). Resveratrol is the parent compound of a family of molecules including glucosides and polymers, existing in *cis* and *trans* configuration in narrow range of spermatophytes of which vines, peanuts and pines are the main representatives (Soleas et al., 1997). Resveratrol has been found in at least 72 plant species, and a number of the human diet, such as mulberries, peanuts and grapes. Relatively high quantities are found in the latter (Dercks and Creasy, 1989). Fresh grape skin contains about 50 to 100 mg of Resveratrol per gram, and it is concentration in red wine is in the range of 1.5 to 3 mg/l (Jeandet et al., 1991). Resveratrol, possess diverse biological activities that confer protection against oxidative stress, inflammmation, aggregate functions, cardiovascular disease, and cancer (Baur et al., 2006; Kris-Etherton et al., 2002; Athar et al., 2007; Shankar et al., 2007; King et al., 2005). Aside from cardiovascular disease, resveratrol has been reported to potentially benefit a number of conditions, including cancer (Kaminski et al., 2011). Resveratrol has received a great deal of attention because it blocks the multistep process of carcinogenesis at various stages: carcinogen activation, tumor initiation, tumor promotion, and tumor progression (Jang et al., 1997). Resveratrol suppresses proliferation of a wide variety of tumor cells, including lymphoid,

myeloid, breast, prostate, stomach, colon, pancreas, thyroid, skin, head and neck, ovarian, and cervical. It has been demonstrated to inhibit carcinogenesis by acting as an antioxidant, anti-inflammatory, antimutagen, antimetastatic, antiangiogenic, antiproliferative and pro-apoptottic agent. It modulates signal transduction, the immune response, transcription factors, growth factors, cytokines, caspases, interleukins, prostaglandin synthesis and cell cycle-regulating proteins. Resveratrol sensitizes chemotherapy-resistant lymphoma cells to treatment with pactitaxel-based chemotherapy (Fulda, 2002). Moreover, trans-resveratrol appears to protect against diabetes (Sharma et al., 2007) and neurodegenerative disorders (Tredici et al., 1999). Experimental studies have shown that resveratrol exhibits both anti-inflammatory and cardio-protective potential by inhibiting the expression of inflammatory mediators and the monocyte adhesion to vascular endothelial cells (Carluccio et al., 2007; Csiszar et al., 2006). Although resveratrol exhibits potent anticancer activities against transformed cells, its effectiveness is limited by it is poor bioavailability and as a dietary phytonutrients it is most effective against tumors with which it comes in direct contact, such as skin cancers and tumors of the gastrointestinal tract. Furthermore inhibition of sirtuin 1 by both pharmacological and genetic means abolished protein de-acetylation and autophagy as stimulated by resveratrol, but not by piceatannol, indicating that these compounds act through distinct molecular pathways. In support of this notion, resveratrol and piceatannol synergized in inducing autophagy as well as in promoting cytoplasmic protein de-acetylation (Pietrocola et al., 2012)

I.6. Impact of winemaking techniques on wine polyphenols

This part of the literature was published as a chapter book entitled “Impact of winemaking technique on phenolic compounds composition and content of wine: A review” (Chapter book published in phenolic composition, classification and health benefits, Nova Science publishers, Inc, 2014) (Ghanem et al., p.103-130).

I.6.1. INTRODUCTION

Phenolic compounds or polyphenols represent a large group of molecules which are present in grapes and wines. These compounds constitute a decisive factor in red wine quality and contribute to wine organoleptic characteristic such as color, taste, astringency and bitterness. They also confer to the wine the capacity of aging. The chemical composition of these

compounds is discussed in other chapters. The antioxidant properties of phenolic compounds have been associated with health-promoting effects. Nowadays, the anticarcenogenic ability and the neuroprotective effect of these compounds are slightly proven and still under investigation. Scientific papers showed clearly that several factors affect polyphenols biosynthesis and accumulation through berry ripening (Spayd et al., 2002; Zoecklein et al., 2008; Poni et al., 2009). Among these factors, the cultivars varieties (clones, and rootstock), the environmental factors (agro-pedological, topographical and climatic factors) and the cultural practices (training system, row vine spacing, pruning, bunch thinning, bud and leaf removal, water, fertilizers and pesticides management) play a crucial role in the determination of the quantitative as well as the qualitative phenolic composition.

After grape harvest, the winemaking process begins. Regardless the geographical zone, the winemaking process scheme is almost the same with some steps modification. The general scheme of winemaking is presented in Figure I.16. During this process, the diffusion and extraction of the grape polyphenols take place and a perpetual evolution of the phenolic composition of the must at the beginning and of the wine later, occurs with the participation of biochemical and chemical phenomena. New technologies and processes (membrane processes, flash release, etc) have been introduced to wine industry in response to various challenges as climate change, wine with low alcohol content, better quality, higher production, new products etc. It is obvious that the traditional and new processes hugely impact the qualitative and quantitative composition of phenolic compounds.

Therefore, in this chapter, we will review the impact of traditional and new processes of winemaking on wine phenolic composition. It will be focused on the incidence of maceration type and time, fermentation process, aging step, finning and clarification methods, membrane processes and filtration techniques as well as the microoxygenation step on wine polyphenols. The factors influencing the grape polyphenols content will not be discussed despite their importance.

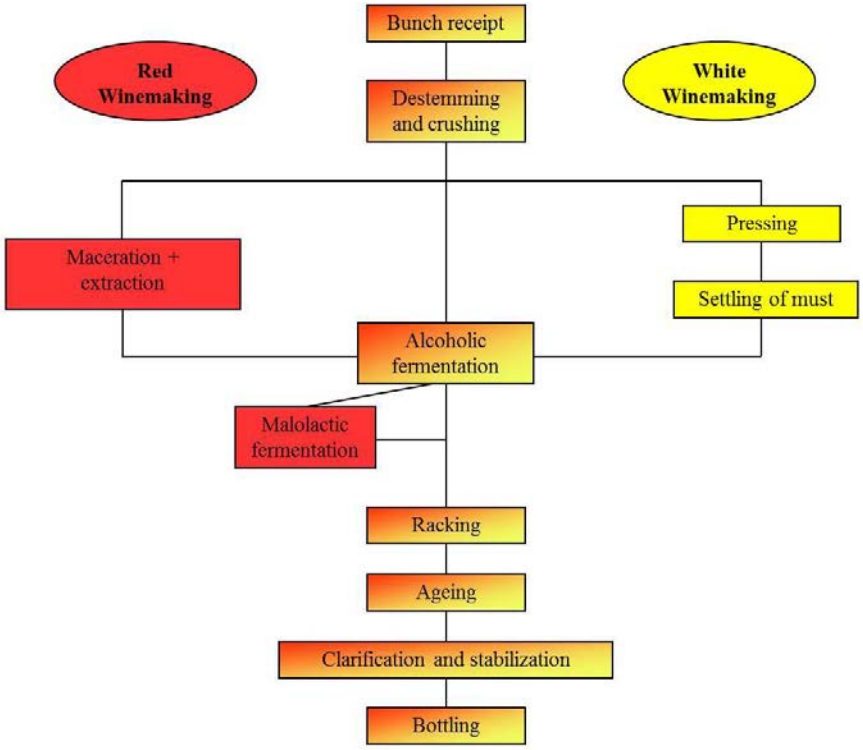


Figure I.16: The winemaking process of red and white wines

I.6.2. IMPACT OF EXTRACTION PROCESSES AND PROCEDURES

Most grape phenolics are localized in the skins and seeds. During winemaking, phenolic compounds and other compounds contained in the grape are transferred to the wine by diffusion while the contact between the juice and the solid part of grapes is established.

Diffusion is the process by which a compound moves from a region of high concentration toward a region of lower concentration. The diffusion period in winemaking is called maceration and it is affected by several factors as grape variety and maturity, temperature of must or wine, pumping over wine, duration of juice and grape skin and seed contact, concentration of alcohol and sulfur dioxide and use of enzymes.

In order to extend the extraction that occur during conventional maceration, and to achieve organoleptic properties beyond those offered by conventional maceration during fermentation, extended contact with skins may occur before (pre-fermentation extended maceration) or after fermentation (post-fermentation extended maceration). Depending on the temperature levels, the

pre-fermentation extended maceration could be divided into two categories: i) cold maceration or cold soak for low levels of temperature, ii) heating maceration.

The effect of cold soak technique as regards with control vinification on the concentration of anthocyanins and proanthocyanidins in Monastrell wine was studied by Busse-Valverde et al. (2010) and the results have shown an increase in the anthocyanin extraction, mainly the extraction of malvidin-3-glucoside with higher grade of co-pigmentation and polymerization, therefore better stabilization than traditional wines. Gómez-Míguez et al. (2006) and Gordillo et al. (2010) reported a similar anthocyanin concentration after seven days of low temperature pre-fermentation maceration of Syrah and Tempranillo wine. Gil-Muñoz et al. (2009) found that cold maceration technique led to the highest anthocyanin content at the end of alcoholic fermentation in Cabernet Sauvignon wines. Consequently cold macerated wines tended to show higher chromatic stability than traditional maceration (Gordillo et al., 2010). When the results of the different practices were compared in Monastrell wines (Busse-Valverde et al., 2010), the proanthocyanidins concentration was greatest when cold soak was used (an increase of 33% in the proanthocyanidins concentration). In Cabernet Sauvignon wines, the proanthocyanidins content was higher when cold soak was used, with a total increase of 13.2%. Alvarez et al. (2006) also found a positive effect of low temperature pre-fermentative maceration on the concentration and polymerization of proanthocyanidins and on the stability of Monastrell wine color. Cold soak increased the ratio of anthocyanin to proanthocyanidins (67%) in the wine after maceration, suggesting a possible increase in the proportion of the anthocyanin-proanthocyanidin adducts against total polymers, which must affect the quality of the resultant wine after long-term storage (Cheynier et al., 1999).These authors also stated that the phenolic concentration was not related to the duration of the treatments since the results did not improve when pre-fermentation maceration time was increased but the effect was more evident when grapes were not completely mature. Alvarez et al. (2006) found an important decrease of the proanthocyanidins, of the mean degree of polymerization (mDP) and of the percentage of epigallocatechin (EGC) and an increase of the percentage of galloylation were reported when cold soak maceration was used. It might be expected that, with the application of these low temperature techniques, which are supposed to help the physical degradation of skin cell walls, the concentration of skin proanthocyanidins would increase in the wines, but these results indicated that the increase in proanthocyanidins is mainly due to an increase in seed

proanthocyanidins. These results also agree with those obtained by the study of Busse-Valverde et al. (2010) who showed that proanthocyanidins concentrations in Monsatrell and Cabernet Sauvignon wines are increased with cold treatment and this increase seems to be related to the extraction of seeds proanthocyanidins. The cold Treatment is more effective when it is realized with less mature grapes (Álvarez et al. 2006). This result is according to previous studies for wines of other grape varieties (Couasnon, 1999).

I.6.3. PRE-FERMENTATION HEATING MACERATION

Historically, this type of maceration is coupled to the fermentation in liquid phase. Practically, it is used to quickly handle the entry of large volumes of grape harvest. Technically, this practice is used to extract phenolic compounds, denature alteration enzymes and destruct vegetal aromas of grapes. Many variations of pre-fermentation heating maceration exist:

i) The pre-fermentation heating maceration followed by direct pressing: grapes are heated to 70-75°C. The maceration must last between 6 to15 hours to obtain the same amount of polyphenols as a classical vinification (Temperature between 25°C-30°C for 3 to 21 days)

ii) The pre-fermentation heating maceration followed by maceration during fermentation: its principle is the same as the first pre-fermentation heating maceration but heating maceration lasts 2 hours and then the bunch is cooled down.

iii) The thermo-vinification process: this technique consists in bunch heating to 70-75°C for a short duration (30-40 minutes). The bunch is then pressed and cooled. Wines are generally less rich in phenolic compounds comparing to a classical vinification (Sacchi et al., 2005)

iv) The flash release (FR) process: it consists in heating the grapes quickly at high temperature (>95°C) with biological vapor (i.e., steam produced from the water present in the grape, without dilution) at atmospheric pressure and then placing them under a strong vacuum (pressure closed to 60 hPa) which causes instant vaporization. The vaporization induces weakness in the cells wall and cooling of the treated grapes which favorites the polyphenol extraction. It is generally coupled to fermentation in liquid phase.

In 2000, Berger and Cottereau studied the winemaking of fruity red wines by pre-fermentation maceration under heat. The trials were conducted in the Beaujolais using a pre-fermentation heating to 70°C lasting from 8 to 16 hours. They found that this technique increase significantly the color (+40%) and tannins content (+55%) comparing to traditional Beaujolais vinification. It was also judged that the pre-fermentation technique influenced the wine aromas with red fruits notes.

The influence of bunch heating technique on the phenolic composition of red wines (Pinot noir, Lemberger and Cabernet Franc), regarding those of the control wines, was studied by Netzel et al. (2003), and the results have shown an efficient extraction of anthocyanins (located in the skin of red grapes), flavonols (especially quercetin glycosides accumulate in the skin), resveratrol (stored within the grape cells in the form of glucosides) and total flavan-3-ols (highest concentration in the seeds), while the level of individual monomeric (catechin and epicatechin) and dimeric (proanthocyanidins B1 and B2) flavan-3-ols were similar to or less than the control wine. These results were in accordance with those obtained by Borazan and Bozan (2013). In contrast, the phenolic acids (found in the skin, juice, solid pulp, and seeds) and tyrosol (produced from tyrosine by yeast during fermentation which is the only phenolic compound produced in significant amounts from non-phenolic precursors) did not show these effects.

A preliminary study carried out on flash-release (FR) by Moutounet et al. (2000) showed an increase of 50% in the total phenolic compounds than that observed in the control wines. In 2006, Morel-Salmi et al. applied FR treatment on three grape varieties in different vintage (Grenache, Mourvedre, Carigan), and the results, presented in Table I.1, showed that FR wines contained larger amounts of flavonols, anthocyanins, catechins and proanthocyanidins (tannins) than the control wines. The average chain length of proanthocyanidins (mean degree of polymerization, mDP) in control and FR wines were almost identical. The FR-treated wines contained higher percentages of galloylated units and lower proportions of epigallocatechin (EGC) units than the control wines (Table I.1). Again, this presumably reflects the fact that the extraction of tannins from seeds is greater than that of the skins (Morel-Salmi et al., 2006). This study also showed that FR increased total anthocyanins, total polyphenol index (TPI), color intensity (CI) and it lowered sulfite bleaching resistance than in the corresponding control wines. FR increased the tannin-to-anthocyanin ratio. This increase in the ratio T/A allows the conversion of anthocyanins to T-A dimers adducts that show the same color properties as

anthocyanin. Formation of T-A adducts increased with the oxygenation, tannin-to-anthocyanin ratio and with FR and heating (Fulcrand et al., 2004).

Table I.1: Effect of Flash Release on the Wine Polyphenol and Proanthocyanidin Composition (mg/l) (Morel-Salmi et al., 2006)

		Anthocyanins	Flavonols	Hydroxycinnamic acids	Catechins	Proanthocyanidins	DPm	% gall	% EGC
Grenache 2003	control	106.1 ± 8.6	20.8 ± 1.4	460.8 ± 12.8	85.2 ± 12.8	751.2 ± 46.9	4.00 ± 0.16	3.42 ± 0.16	10.51 ± 0.4
	flash release	110.9 ± 2.1	30.8 ± 1.1	355.7 ± 7.3	143.3 ± 1.9	997.2 ± 59.1	3.3 ± 0.13	4.8 ± 0.3	7.52 ± 0.3
Mourvèdre 2003	control	173.2 ± 4.7	36.1 ± 2.0	47.9 ± 5.8	33.9 ± 1.4	819.5 ± 52.1	4.78 ± 0.18	3.1 ± 0.17	13.9 ± 0.6
	flash release	198.7 ± 1.7	67.9 ± 0.3	26.8 ± 1.4	46.8 ± 3.1	1281.7 ± 308.5	4.5 ± 0.2	4.9 ± 0.3	9.5 ± 0.3
Grenache 2004	control	83.5 ± 0.5	5.4 ± 0.6	316.1 ± 5.4	55.4 ± 2.6	564.1 ± 35.2	3.95 ± 0.13	4.04 ± 0.28	12.1 ± 0.85
	flash release	87.6 ± 0.7	9.4 ± 0.1	270.8 ± 5.7	113.8 ± 3.4	851.5 ± 36.0	3.11 ± 0.06	5.3 ± 0.16	7.5 ± 0.07
Carignan 2004	control	161.4 ± 1.9	13.4 ± 1.2	94.1 ± 3.8	26.0 ± 0.7	308.7 ± 9.2	3.97 ± 0.07	2.08 ± 0.1	19.0 ± 0.66
	flash release	210.3 ± 5.6	27.5 ± 0.7	103.2 ± 2.8	32.3 ± 0.8	356.2 ± 11.2	4.02 ± 0.12	3.0 ± 0.12	17.6 ± 0.2

I.6.4. CARBONIC MACERATION

Carbonic maceration consists of placing whole grapes in a closed tank under CO_2 atmosphere. The tank is kept at a moderate temperature (20-30°C) for 1-2 weeks. The carbon dioxide gas permeates through the grape skins and begins to stimulate fermentation at an intracellular level. The entire process takes place inside each single, intact berry. Then the juice is run off, the pomace pressed, and the free- run and press wines are usually assembled prior to normal alcoholic and malolactic fermentation (Ribéreau et al., 2006).

The influence of fermentation with carbonic maceration, on the contents of catechins, proanthocyanidins, and anthocyanins in Tinta Miúda red wines, was studied by Sun et al. (2001). They reported that the carbonic maceration wine contained the highest amounts of catechins, oligomeric and polymeric proanthocyanidins comparing to traditional process of winemaking, which might be explained by the fact that the phenolic compounds released from the solid parts of the grape cluster, using the carbonic maceration technique, are well-protected against oxidation or other physicochemical reactions during intracellular fermentation/maceration (Sun et al., 2001). On the other hand, analysis of individual and total anthocyanins by Sun et al. (2001) has shown that the concentrations of total anthocyanins and nearly all individual anthocyanins in the carbonic maceration wine were lower than traditional wine. Moreover, the carbonic maceration wine had less colored density and higher hue than the control wine.

Sun and Spranger (2005) also reported highest procyanidin levels in carbonic maceration Tinta Miúda red wines. It was shown also that carbonic maceration afforded wines with most stability in color density for 26 months‟ storage. On the opposite, Spranger et al. (2004) detected higher catechins and procyanidin levels in Castelão red wines made by classical techniques. Castillo-Sanchez et al. (2008) also found that procyanidin and catechin levels in traditional wines were much higher than in carbonic maceration wines

While studying the influence of winemaking protocol on the evolution of the anthocyanin content, Castillo-Sanchez et al. (2006) showed also that carbonic maceration led to lower anthocyanin levels and less intense coloration than the conventional pumping over and the rotary vats. They claimed also that during storage, the carbonic maceration wines underwent less color degradation than the others.

Castillo-Sanchez et al. (2008) found that carbonic maceration produced wines with less color density and higher hue than the conventional process of winemaking. These results were in

agreement with those obtained by Timberlake and Bridle (1976). Although, the carbonic maceration protocol might have been expected to increase the release of anthocyanins from the grape skin due to the longer overall time spent macerating and fermenting and to the higher temperature used (Lorincz et al., 1998), these effects seem to have been overweighed by the effect of reducing post-crushing fermentation time to 2–3 days, which reduced the duration of intimate contact between skin and must. Similar results were obtained by Spranger et al. (2004) where they detected higher anthocyanin levels in classical fermentation Castelão red wines obtained than in carbonic maceration Castelão red wines.

I.6.5. POST-FERMENTATION RE-HEATING

It is a method consisting in prolonging the fermentative maceration by post-fermentation re-heating to approximately 45°C for 42 hours, in order to complete the liberation of grape skin constituents fulfilled by pre-fermentative and fermentative maceration. Discordant results are reported in literature on the effect of post-fermentation re-heating on red wine quality. A study by Koyama et al. (2007) on the influence of heating at the end of maceration during red winemaking from Cabernet Sauvignon showed that, contrary to expectations, the anthocyanin concentration was not increased. Flavonols showed an extraction similar to anthocyanins, while heat treatment decrease the level of proanthocyanidins, their mDP and the galloylation rate (%G). Barra et al. (2005) obtained higher increase in anthocyanin content (+10.7% of malvidin 3-glucoside), in color intensity (+13%) and total sensory score, in Pinot noir wine by heating at the end of maceration than by control vinification. Similar results were reported by Gerbaux et al. (2003). Potential variables, e.g., grape variety, berry maturity, heat conditions and fermentation scale might have affected the results.

I.6.6. MACERATION ENZYMES

The grape skin cell walls formed mainly by polysaccharides (pectins, hemicellulose and cellulose) are limiting barrier that prevent the release of polyphenols into the must during fermentation. Maceration enzymes may help in phenolic extraction and, at the same time, may modify the stability, taste and structure of red wines, because it is not only anthocyanins that are released from skins, but also tannins bound to the cell walls. These may be extracted due to the action of pectinases (polygalacturonase, pectin lyase and pectin esterase activities),

hemicellulases and cellulases and the extracted compounds help to stabilize wine color and increase mouth feel sensations (Canal-Llaubères and Pouns, 2002). In the literature, the effect of the addition of enzymes on the phenolic content remains unclear because of some contradictory results.

The effect of enzymes treatment on phenolic composition was tested on a Monastrell wine by Bautista-Ortín et al. (2007) and it was compared to two other enological practices (running-off a part of must and tannins addition). The authors noticed that the addition of enzymes (pectinase + mannanase + glucanase activities) promoted higher values of total phenols (OD280) than in the control wine as also observed by Parley (1997) and Pardo et al. (1999). It seems that the action of the enzyme facilitates a higher extraction of proanthocyanidins from both skin and seeds but without changing their proportion or composition, as compared to control wines (Busse-Valverde et al., 2011). On the opposite, Ducasse et al. (2010) found a higher proanthocyanidin content in Merlot wines treated with enzymes but, surprisingly, not in the percentage of skin-derived proanthocyanidins, but with an increase of seed proanthocyanidins. Regarding anthocyanin concentrations, some authors have reported an increase in the anthocyanin levels (Bautista-Ortin et al., 2005; Kammerer et al., 2005; Romero-Cascales et al., 2012), whereas others have reported a decrease in the anthocyanin levels (Kelebek et al. 2007; Parley et al., 2001; Revilla and Gonzales-Sanjose, 2003). Borazan and Bozan (2013) studied also the effect of pectolytic enzymes on the phenolic composition of Okozguzo wines. They found that the wines treated by the pectolytic enzyme addition had a lower monomeric flavan-3-ol content than the untreated wines, and that the amount of monomeric anthocyanins extracted did not increase with the addition of enzymes.

This different observations could be due to a different activities present in enzyme commercial preparations.

I.6.7. EFFECT OF YEASTS AND BACTERIA

Yeasts and bacterial metabolism during fermentations produce a large array of metabolites which contribute to the aroma and flavor of wine.

The influence of yeast used for winemaking on phenolic compounds is still poorly understood; but it is known that yeasts interact with polyphenols by 3 mechanisms:

- Adsorption of phenolic compounds on yeast cell wall

- Extraction of phenolic compounds
- Excretion of parietal polysaccharides (mannoproteins) which can interact with tannins for better stabilization and sensorial perception of the wine.

Hayasaka et al. (2007) studied the impact of two different yeasts, *Saccharomyces cerevisiae* (SC) and *Saccharomyces bayanus* (SB) on the phenolic composition of red wine made from the same batch of Cabernet Sauvignon grapes. The color properties and pigment profiles of SC and SB wines were compared at 8 days and 387 days after yeast inoculation. Anthocyanin concentration was found to be lower in SB wines than in SC wines at day 8 and 387, but SB wine exhibited greater wine color density. The anthocyanin concentration did not correlate with wine color density. The levels of pigmented polymers and SO_2 non-bleachable pigments were found to be higher in SB wine at day 387, demonstrating that the formation of stable pyranoanthocyanins and pigmented polymers was enhanced by SB yeast. It was demonstrated that the formation of acetaldehyde-mediated pigments was enhanced by the use of the SB yeast. The compositional analysis suggested that the differences in color properties and pigment profiles of SC and SB wines were largely due to the greater production of acetaldehyde-mediated pigments by the use of SB yeast.

Caridi et al. (2004) studied the effect of two yeasts strains on the phenolic profile of red wine. They reported that the Strain Sc2659, compared to strain Sc1483, produced a wine with significantly higher values of color, color intensity, total polyphenols and monomeric anthocyanins. Also, the content of flavonoids, total anthocyanins, flavans and proanthocyanidins was higher in the wine produced by strain Sc2659, but the differences from the strain Sc1483 were not significant. The levels of non- anthocyanic flavonoids were significantly lower. Therefore, strain Sc2659 protects during winemaking the phenolics and the anthocyanins of the must better than strain Sc1483.

Two commercial yeast strains (Fermirouge and Rhône 2323) were tested during the winemaking process of Monastrell grapes to determine their influence on color and phenolic composition of the resulting wines during alcoholic fermentation and maturation. The results showed that in 2002, the wines did not present great differences but in 2003 higher color intensity and phenolic compounds content were detected when one of the commercial strains was used. The maximum values of monomeric anthocyanins were found when Rhône 2323 (L2) was used. In 2003, differences in hydroxybenzoic acids, flavan-3-ols and total anthocyanins were also found. Rhône

2323 (L2) wines presented the largest concentration of these compounds (Bautista-Ortin et al., 2007).

Yang Sun et al., (2011) studied the effect of six commercial wine yeast strains (BM4x4, RA17, RC212, D254, D21 and GRE) on the profiles of polyphenols in cherry wines. They showed that BM4x4 fermented wine had the highest total phenolics and tannins among the six wines tested, whereas RC212 fermented wine had the highest content of total anthocyanins. Therefore a wide range of concentrations of total anthocyanins, total phenolics and tannins were revealed depending on yeast strains.

Regarding low molecular weight phenolic compounds, it is known that some phenolic acids can inhibit the growth of lactic acid bacteria while others can stimulate malolactic fermentation carried out by *Oenococus oeni*. During this process, hydroxycinnamic acids and their derivatives are the main compounds modified. The decrease in the concentration of *trans*-caftaric and *trans*-*p*-coutaric acids until disappearance, along with an increase in the corresponding free forms, *trans*-caffeic and *trans*-*p*-coumaric acids could be linked to lactic acid bacteria metabolism.

It has been described that *Lactobacillus hilgardii* can degrade gallic acid and catechin (Alberto et al., 2004). *Pediococcus pentosaceus* can also reduce the quercetin levels (Locascio et al., 2006). *Oenococcus oeni* was found to be able to metabolize anthocyanins and other phenolics by a glycosidase action producing important wine aroma compounds (De Revel et al., 2005; Bloem et al., 2008).

Bloem et al. (2006) studied the production of vanillin from simple phenols by wine-associated lactic acid bacteria. They found that bacteria were not able to form vanillin from eugenol or vanillic acid. However, they showed that *Oenococcus oeni* could convert ferulic acid to vanillin.

Cabrita et al. (2008) reported that hydroxycinnamic acids and their derivatives were the main compounds modified by malolactic fermentation, independently of the use or not of commercial lactic bacteria. In fact, it seems clear that the decrease in the concentrations of caftaric, coutaric and fertaric acids, and the increase in the concentrations of caffeic, *p*-coumaric and ferulic acids are linked to lactic acid bacteria metabolism.

I.6.8. REACTION BETWEEN ANTHOCYANINS AND TANNINS: IMPACT OF MICRO-OXYGENATION

Anthocyanins are the most significant components, responsible for the purple-red color of young red wines. They are unstable and participate in reactions during fermentation and maturation to

form more complex pigments, which mainly arise from the interaction between anthocyanins and other phenolic compounds, especially flavan-3-ols. Several mechanisms have been proposed and confirmed for the formation of these new pigments:

a) Direct anthocyanin-tannin condensation reactions (A^+-T product). The products are colorless flavenes, which can be oxidized to the corresponding flavylium ions, finally developing into yellow xanthylium salts. These reactions take place during fermentation, and O_2 is required (Liao et al. 1992; Santos-Buelga et al. 1999; Ribéreau-Gayon et al. 2006).
b) Direct tannin-anthocyanin condensation reactions (T^+-A). The products are colorless, but are rapidly dehydrated into a reddish-orange form. This reaction is stimulated by higher temperatures, and O_2 is not required. It occurs predominantly during bottle aging (Remy et al. 2000; Ribéreau-Gayon et al. 2006; Hayaska and Kennedy, 2003).
c) Reactions between anthocyanins and flavanols mediated by acetaldehyde to give a resulting product, with an ethyl bond, that can be protonated to form a colored compound. (Timberlake and Bridle, 1976; Francia-Aricha et al., 1997). Acetaldehyde can be derived from ethanol oxidation or from yeast metabolites.
d) Cycloaddition reactions to form pyranoanthocyanin compounds. Anthocyanins react with yeast metabolites or wine oxidation products (e.g. vinyl phenols, acetaldehyde and pyruvic acid). Vitisin-B is the specific compound resulting from ethenol (aldo-enol transformation of acetaldehyde) and malvidin-3-glucoside. Phenylpyranoanthocyanins, carboxypyranoanthocyanins and pyranoanthocyanins are respectively the results of the reaction between anthocyanins and vinylphenols, pyruvic acid and acetaldehyde (Atanasova et al., 2002; Mateus et al., 2003; Fulcrand et al., 2006; Rentzsch et al., 2007).
e) Addition reactions between anthocyanins and oxidized phenolic compounds (i.e. ortho-quinones) (Cheynier, 2006; Guyot et al. 1996).
f) Depolymerization and repolymerization reactions of tannins during wine aging. These transformations can occur in the presence or absence of oxygen; however, the resulting structures will differ, depending on the pathways taken (Vidal and Aagaard, 2008). Oxygen brings about the production of different aldehydes, with acetaldehyde being the most abundant. Subsequently, acetaldehyde can react rapidly with tannin molecules. The

resulting products are not as important as are direct C4–C8 and C4–C6 polymerization reactions between procyanidin molecules and are hence less astringent (Ribéreau-Gayon et al., 1983; Tanaka et al., 1994).

g) Copigmentation of anthocyanins. The phenomenon of copigmentation is due to molecular association between anthocyanins (intramolecular copigmentation) or between anthocyanins and other non-colored organic molecules (intermolecular copigmentation). Copigmentation is important in color modification in young red wines, promoting an increase in the maximum absorption wavelength

All of these reactions result in the formation of more stable compounds that stabilize wine color since they partly resist discoloration by SO_2 and provide better color stability at wine pH.

Micro-oxygenation (MOX) is a technique that consists in introducing small and measured amounts of oxygen into wines with the objective of improving wine color, aroma and texture and involves the use of specialized equipment to regulate the oxygen doses applied (Parish et al. 2000; Paul, 2002). The term does not usually include the passive oxygen exposure that occurs during barrel aging nor the range of winemaking practices (such as pumping over and racking) where oxygen exposure may be intentional but is not well measured (Rieger, 2000). An important stipulation of micro-oxygenation is to introduce O_2 into the wine at a rate equal to or slightly less than the wine‟s ability to consume that, avoiding accumulation of dissolved oxygen (Du Toit et al. 2006). It is for this reason that the success of MOX depends strongly on controlling the rate of oxygen exposure. Typical dosage rates are relatively small, ranging from 2 to 90 mg O_2/l of wine/month (Dykes, 2007). Studies on MOX applications indicate that it can be performed at any time during the winemaking process. However, the best results are achieved when oxygen is added at the end of alcohol fermentation and before beginning malolactic fermentation (Parish et al., 2000; Castellari et al., 1998, González-Sanjosé et al., 2008).

A study conducted by Sánchez-Iglesias et al. (2009) on the effect of MOX on the phenolic fraction of Tempranillo wines during two consecutive vintages, showed significant higher contents of total anthocyanins, pyruvic derivates and polymerization pigments than the control wines, in which most of the pigments belonged to the group of flavanol-anthocyanin (direct and ethyl-bridged) derivatives (Arapitsas et al. 2012). Similar results were observed by (Atanasova et al. 2002) in blended red wine (var. Cabernet Sauvignon and Tannat) with a decrease in the

percentage of copigmentation. As regards of chromatic parameters all of the micro-oxygenation wines showed significantly higher values of color intensity and percentage of blue, with a lower percentage of red and yellow than the control wines (Sánchez-Iglesias et al. 2009). These data agree with those already described for the greater anthocyanin drop, together with higher percentages of polymeric anthocyanins and greater contents of pyruvic derivatives (Revilla et al. 2001; Revilla et al. 2002). On the other hand a study carried out by Cejudo-Bastante et al. (2011) on the effects of micro-oxygenation before malolactic fermentation on Cencibel red wines, showed a decrease of the content of flavan-3-ols versus non micro-oxygenation wines. The micro-oxygenation treatment, together with the aforementioned lower content of flavan-3-ols, suggests that the oxygen addition activated the reactions between free anthocyanins and flavan-3-ols. As a consequence, new anthocyanin-derived pigments more stable to pH changes and bisulphite bleaching were formed (Escribano-Bailón et al. 2001). The latter was supported by the increase of percentage of polymerization and the lower value of copigmented anthocyanin (Hermosín-Gutiérrez et al. 2005).

The formation of polyphenolic compounds and pyranoanthocyanins during MOX could be enhanced by the presence of oak. This latter contains high amounts of hydrolyzable tannins such as ellagitannins and gallotannins. These compounds have high gallolated content that is more efficiently oxidized than the majority of the grape-derived phenolic compounds which are non-gallolated (Schmidtke et al., 2011).

I.6.9. BARREL AGING

Aging in wooden barrels is a process used to stabilize the color and enrich the sensorial characteristics of wine. Many compounds are released from wood into the wine; oxygen permeation through the wood favors formation of new anthocyanin and tannins derivates (De Rosso et al., 2009). During barrel aging, the total anthocyanins monoglucosides (the monoglucosides of delphinidin, cyanidin, peonidin, petunidin and malvidin, together with their acetyl and coumaryl derivates) decreased, but the percentage of pigments in the red form increased from 15 to 45%. This transformation of colorless anthocyanins (free anthocyanins) into the colored form (polymerized compounds) compensates for their loss and leads to the increase in color density (Cano- Lopez et al., 2010). On the other hand, a drop in free and total anthocyanins was thus observed, with the concentration of anthocyanins dropping from about

850 mg/l to 400 mg/l within six months (Atanosova et al., 2002). The concentration of direct adducts (T-A^+ or T^+-A) increased after six months in new barrels (Cáno-López et al., 2010). As regards their chromatic characteristics the color intensity (the sum of the yellow, red and blue colors) increased from 8 to 10 and 12 to 16 between 3-6 months after barreling. The percentages of red color was lower than that of the control wine but the percentages of yellow and blue were higher due to pigments resistant to SO_2 discoloration. Such a difference in color density can be observed visually. In South African Pinotage and Shiraz wines, it was found that the origin of the barrel (American, French or Russian) did not affect the difference in color intensity, color hue or total red pigments. The Total phenol content (expressed as optical density at 280nm) increased in barrel aged wines as regards of the control wine due to the extraction of phenolic compounds from oak (phenolic acids, ellagitannins, wood aldehydes) (Gómez-Cordoves et al., 1995).

Several of these positive modifications in wine phenolics occurring during wood aging are due to: (1) the release of ellagitannins from wood to wine. These compounds have 15 OH groups per molecule and are highly reactive toward oxygen penetrating through wood. In the presence of oxygen, the ellagitannins will be more easily oxidized than the majority of grape constituents such as anthocyanins to produce hydrogen peroxide. When hydrogen peroxide reacts with ferrous iron to yield the hydroxyl radical, this highly unstable radical reacts almost immediately. It does not react selectively with anti-oxidants such as phenolics, but instead reacts with all substances present in solution, almost in proportion to their concentration (Gómez-plaza and Cano-López, 2011). Expected products in wine would be the oxidation of alcohol to acetaldehyde (Wildenradt et al., 1974), molecule that is incorporated into red wine phenolic polymers (Drinkine et al., 2007). As a consequence, a modification of red wine color (Timberlake et al., 1976) occurs. The phenolic compounds released from wood may also directly interact with colorant matter of wine giving condensation products bringing to a bathochromic shift of color absorbance (Quideau et al*.,* 2005). (2) Condensation reactions occur between wine phenolics and aldehydes released from oak barrels (Es-Safi et al., 2000; Sousa et al., 2005). In this regard, it has been recently shown that the vanillin, one of the main aldehydes released from oak wood, leads to the formation of an anthocyanin–catechin purple pigment (Sousa et al., 2007). Due to the fact that some acetaldehyde-derived flavanol–anthocyanin polymers are insoluble (Escribano-Bailon et al., 2001), a precipitation of phenolics also occurs. According to several

authors, this might explain the losses of astringent material observed as a result of wood aging (Haslam et al., 1980; Vivas et al, 1996). Another mechanism that has to be considered is the adsorption of wine phenolics on wood. In a study performed using a model solution, it has been observed that at least 50% of the resveratrol content can be sorbed by the wood (Barrera-Garcia et al*.,* 2007), indicating that the wood sorption process was selective for the most hydrophobic compound. Different phenolic molecules are involved with the bitterness, astringency and fullness of red wine, but it is mainly the flavanols that are responsible for these tastes and flavors. A very young red wine might be harsh, course, very astringent and even bitter. During aging of red wine in barrels the wine becomes softer and less astringent. It is mainly the acetaldehyde-induced polymerization that contributes to the polymerization of flavanols. The resulting products are not as reactive towards proteins as their constituents. However, direct C4-C8 and C4-C6 polymerization reactions between procyanidin molecules produce products that are more reactive towards proteins and are hence more astringent than those formed from acetaldehyde-induced condensation reactions (Cheynier et al., 1997). In the case of flavanols, where the C6 and C8 positions can be occupied, polymers larger than trimers have been isolated. Both types of reactions produce procyanidins with a limit of 8 or 10 flavan units. The interaction of anthocyanin molecules with procyanidins can also influence the taste of wine because they can form the terminal subunits, thus preventing further polymerization (Ribéreau-Gayon et al., 2006; Monagas et al., 2005).

I.6.10. AGING ON LEES

The definition of wine lees given by EEC regulation No. 337/79 states that „„wine lees is the residue that forms at the bottom of recipients containing wine, after fermentation, during storage or after authorized treatments, as well as the residue obtained following the filtration or centrifugation of this product" (Pérez-Serradilla and Luque de Castro, 2008). When wine is kept in contact with lees, the yeast covering is naturally and slowly degraded and most nutrient supplies are depleted. This microbiological phenomenon, known as autolysis, is mainly induced through different enzymatic activities of the yeast itself. This degradation in wine enriches this latter with products (polysaccharides, peptides and fatty acids) from different cell parts (Mazauric and Salmon, 2005). The importance of wine lees in the aging technique impact on phenolic compound composition comes from the fact that they can adsorb phenolic compounds

and release to wine some compounds, among them enzymes and mannoproteins. The compounds released can influence the structural integration of the wine in terms of phenols, body, aroma and wine stability (Palomero et al., 2009a). Results showed that mannoproteins released during yeast lees autolysis can interact with phenolic compounds, improving the color stability and reducing the wine astringency by decreasing tannin aggregation and precipitation (Feuillat et al., 2000; Poncet-Legrand et al., 2006; Fornairon-Bonnefond et al., 2002).

It has been generally reported that anthocyanins content in wines decreases after contact with lees (Mazauric and Salmon, 2005; Mazauric and Salmon, 2006). This decrease is due to the adsorption of anthocyanins on wine lees. Mazauric and Salmon (2005) showed that this adsorption follows biphasic kinetics: an initial and rapid fixation is followed by a slow, constant and saturating fixation that reaches its maximum after about 1 week. Other authors (Delcroix et al., 1994; Cunier, 1997) explained that anthocyanins decrease during wine aging is due to the degradation of anthocyanins by β-glucosidase enzymes released by yeast lees. Evolution of red wine anthocyanins with or without aging on lees was studied by Moreno-Arribas et al. (2008), and the results showed that wines aged in the presence of lees, had the highest values of anthocyanins-glucosides (delphinidin-3-glucoside, petunidin-3-glucoside, peonidin-3-glucoside, malvidin-3-glucoside) and anthocyanins-cinnamoylglucosides (delphinidin-3-(6‟‟-P-coumarylglucoside), malvidin-3-(6‟‟caffeoylglucoside), malvidin-3-(6‟‟œoumaroylglucoside), than the wines aged without lees. On the other hand, the results showed that the formation of anthocyanin-vinylphenol adducts seems to be favored by yeast and lactic acid bacteria from lees. Similar results were reported by Pozo-Bayón et al. (2004).

One of the disadvantages of aging on lees is that they consume oxygen. Oxygen plays an important role in the stabilization of wine color by enhancing condensation reactions between flavonoids mediated by acetaldehyde and in the cycloaddition reactions between pyruvic acid and anthocyanins. Therefore, the consumption of oxygen can reduce the condensation and polymerization reactions between phenolic compounds and can result in decreasing of wine color. The aging on lees also favors the formation of reduction aromas. It seems that this problem can be resolved by combining the lees and oak aging because oak aging favors the transfer of oxygen into wine.

Hernandez et al. (2006) studied the impact of aging on lees on 38 non-anthocyanin phenolic compounds. They didn‟t observe any significant difference in the content of these compounds

after 14 months of Aging. They reported an increase in hydroxycinnamic acids during aging on lees in oak barrels and they explained this result by the enzymatic activity of yeast and lactic acid bacteria and the hydro-alcoholysis of oak wood. Same results were obtained by Del Barrio-Galan et al. (2012) when studying the effect of the aging on lees on the low molecular weight phenols of Tempranillo red wine aged in oak barrels.

I.6.11. FILTRATION AND MEMBRANE TECHNIQUES

Wines, after alcoholic and malolactic fermentations, are a complex medium and need to be clarified and stabilized. Stabilization could be divided into physico-chemical and microbiological stabilization. The microbiological stabilization is ensured by filtration techniques while the physico-chemical stabilization is achieved through fining process, cold stabilization and addition of stabilizers agents. The filtration methods used in wine industry could be divided into 2 groups:

i) Precoat filtrations using exogenic additives as diatomaceous earth, perlite and cellulose

ii) Filtrations using filtering media as membranes and pads

All filtrations methods have an incidence on the chemical and organoleptic composition of wines (Serrano, 1998). For precoat filtrations, the impact depends on the permeability of the diatomaceous earth. Polyphenols loss in precoat filtrations is noticed through adsorption of the compounds in the exogenic additives.

Polyphenols losses were more studied in cross-flow microfiltration because it was shown that these compounds with polysaccharides are the main responsible of membrane fouling. At the beginning, several authors (Poirier et al., 1985, Belleville et al., 1990 and 1992) have reported that the colloidal deposit on ceramic membranes has an intense red color and therefore they pointed out the implication of polyphenols in membrane fouling. The involvement of wine polyphenols in the membrane fouling was been identified by washing the fouled membrane with acidified methanol. Significant increases in permeability were obtained. This fact can be attributed to the elimination of the layers of phenolic compounds because the other wine constituents are insoluble in this solvent (Cameira Dos Santos, 1996). According to Czekaj et al. (2000) and El Rayess et al. (2011; 2012), an increase in polyphenol concentration in wine leads to a decrease of membrane permeability and thus to an increase of membrane fouling.

Researchers have also demonstrated that membrane materials exhibit different fouling behaviors with wine compounds. Ulbricht et al. (2009) showed that polysaccharides and polyphenols adsorption occurs more on polar (hydrophilic) polyethersulfone (PES) membranes that on non-polar (hydrophobic) polypropylene membranes. In fact, Polyphenols are amphipathic molecules with hydrophobic aromatic rings and hydrophilic phenolic hydroxyl groups. So their adsorption involves both hydrophobic effects and the formation of hydrogen bonds. El Rayess et al. (2012) have reported that the most plausible mechanism for membrane fouling by tannins is a fast interaction between tannins and the ceramic membrane (adsorption) quickly followed by tannins-tannins interaction leading to aggregates that could block the pores and form a deposit at the top surface of the membrane. Recently, it was shown that cross-flow microfiltration significantly decreased the mean degree of polymerization (mDP) of tannins by 25% and it selectively removed the high polymerized proanthocyanidin. It was also reported that this technique lowered the levels of catechin, dimers and anthocyanins comparing to the control (Oberholster et al., 2013).

The effect of other membrane processes (Reverse osmosis, nanofiltration, electrodialysis …) on polyphenol content in wine was also studied. Membrane processes including nanofiltration, reverse osmosis, pervaporation and membrane contactor can be used to reduce alcohol in wines. These techniques form alternatives to traditional techniques. There are two methods to reduce alcohol content in wine: i) reduction of sugar concentration of musts; ii) de-alcoholization of wine (Mietton-Peuchot, 2010). The electrodialysis is used for tartaric stabilization while the bipolar membrane electrodialysis serves to acidify or de-acidify the wine.

Gomez-Benitez et al. (2003) showed a negligible impact of electrodialysis on color intensity. Granès et al. (2009) also demonstrated that bipolar membrane electrodialysis had no effect on polyphenol contents in wine. Cottereau et al. (2010) reported that The REDUX® process (association of ultrafiltration and nanofiltration to reduce the sugar concentration of musts) allows the concentration of polyphenols in wines due to volume reduction. In 2011, Bogianchini et al. evaluated the phenolic profile and the antioxidant activity of commercial dealcoholized wines by reverse osmosis. They found that the reverse osmosis process didn"t significantly affect any phenolic acids regardless to their chemical structure and alcoholic degree but the antioxidant activity decreased in average 40% compared to untreated wine. The antioxidant activities and phenolic compounds of these products were monitored for seven months. No significant changes

were observed. In 2012, Liguori et al. tested the osmotic distillation for wine de-alcoholization and they tested its effect on wine phenolics. No significant differences in chemical analyses between crude and dealcoholized wine were found. The last observation is in agreement with the results obtained by Gambuti et al. (2011) while studying the influence of partial de-alcoholization by membrane contactor on red wines quality.

I.6.12. FINING AGENTS

Fining is used to clarify and stabilize wines. The purpose of fining is to cause haze-forming particles to combine with additional agents, leading to flocculation, clarity, and stabilization. Fining agents are used to eliminate or reduce undesirable substances in wine. Table I.2 summarized the common fining agents, their sources and their applications in enology. Three major mechanisms of action of fining agents include charge-charge (electrical) interaction, bond formation, or absorption/adsorption. Wine components and the type of fining agent determine the mechanism of action. When compounds of opposite charges interact, larger particles form and settle. In the case of bond-formation, chemical bonds (i.e., hydrogen bonds) form between fining agents and wine components. Absorption occurs when compounds are engulfed by the fining agent. Alternatively, when the substance is bound to the agent‟s surface, the substance is adsorbed.

Table I.2: Common fining agents used in winemaking

Fining agent	Source	Purpose of application
Gelatin	Animal Tissue	Removal of tannin and brown polymeric pigments
Isinglass	Fish bladder	Reduce phenolic compounds; add fruitiness to wine
Casein	Milk	Reduce wine haze and tannin content
Egg Albumen	Egg whites	Reduce wine haze and tannin content
Bentonite	Clay, volcanic deposits	Protein removal
Tannin	Wood and grapes seeds	Targets phenolic and proteins compounds
Sparkalloid	Alginate	Clarification and settling aid
Polyvinyl-polypyrrolidone	Synthetic polymer	Reduce polyphenols
Vegetable proteins	Plant proteins	Removal of galloylated and condensed tannin

Winemakers use several chemical substances (Table I.2), the choice of which depends on the nature of the wine and the compounds that are going to be eliminated (Gómez-Plaza et al. 2000). Bentonite is negatively charged clay. The clay consists of complex hydrated aluminum silicate with exchangeable cationic components. Calcium and sodium bentonite are two forms that are commercially available for wine use. The mode of action of bentonite is electrostatic. The flat surface of a hydrated bentonite platelet is negatively charged. Positively charged particles adsorb onto the surface of the bentonite. Bentonite is principally used to remove proteins (protein stabilizer) from white wine and juice. It also attracts other positively charged compounds such as anthocyanins, other phenolics and nitrogen. Bentonite is not reactive towards small phenolic compounds but binds only large phenolic compounds such as anthocyanins and may also bind phenolic compound complexes with protein (Kalkan Yildirim, 2011)

Egg albumin and Gelatin are positively charged proteins used to remove excess negatively charged tannins from wine (Kalkan Yildirim, 2011). They are most commonly used to reduce the level of astringency and bitterness in the press fraction of wines, with reference to soften red wines (Stankovic et al. 2012). Egg albumen is colloidal in nature and has a positively charged surface that attracts negatively charged tannins in red wines. It is unsuitable for white wines

treatment. Whereas, gelatin is primarily used to soften red wines but can also be used to reduce the phenol level and brown color in white juice before fermentation. Gelatin reduces astringency in red wines by lowering tannin levels and tends to remove more, higher molecular weight galloylated proanthocyanidols than lower molecular weight tannins (Sarni-Manchado et al., 1999). After the formation of gelatin-tannin complex, this complex may interact with anthocyanins, causing their removal.

Casein fining preparations are used in particular for the treatment of astringency and for the clarification of white and rosé wines, but are also sometimes used with red wines. Casein is a positively charged protein that flocculates in acidic media such as wine. When added to wine, casein adsorbs and mechanically removes suspended material as it settles. Casein is difficult to mix into the juice/wine as it is relatively insoluble in acidic solutions and should be mixed in water with a pH value above 8 or made alkaline prior to mixing.

Isinglass is a positively charged fining agent derived from the air bladder of a sturgeon. It is available as sheet or flocculated isinglass. Isinglass is used principally in white still and sparkling wines and to clean up the aroma, improve clarity and modify the finish without significantly modifying tannin levels.

Polyvinyl polypyrrolidone (PVPP) is a high molecular weight fining agent made of cross-linked monomer of polyvinlypyrrolidone. It complexes with phenolic and polyphenolic components in wine by adsorption and attracts low molecular weight tannins. It removes bitter compounds and browning precursors in both red and white wines. PVPP is quick acting with no preparation required.

The use of plant-derived proteins as wine fining agent has gained increased interest owing to the potential allergenicity of animal proteins in susceptible subjects. Plant derived proteins (wheat, pea, lentil, soybean and potato…) were effective in giving a fast and remarkable decrease in turbidity. It complexes with high molecular weight tannins by hydrogen bonding. This tannin-protein complex is insoluble and precipitates from the wine (Lefevebre et al., 2003).

Several studies in the literature treated the impact of fining agents on the phenolic composition of wines. A study done by Stankovic et al. (2012), on the effect of fining agents, on red Pinot noir variety of different ages, showed that fined wines lead to significant reduction of color intensity, ionized anthocyanins, and a low reduction of colorless anthocyanins, relative to unfined wines. Castillo-Sanchez et al. (2006) investigated the impact of PVPP, casein, egg

albumin and gelatin on the evolution of anthocyanins and color of Vinhão wines. They found that all fining agents induced loss of color density and anthocyanin content but surprisingly, they noticed that PVPP caused more loss of color than the other fining agents. Several authors also found a decrease in anthocyanin content with fining (Castillo-Sanchez et al., 2008; Cosme et al., 2007; Karamanidou et al., 2011). Cosme et al. (2007) studied the interactions between protein fining agents and proanthocyanidins in white wine. They reported that the monomeric flavanols were significantly depleted by casein, and gelatin with low molecular weight (MW) distribution, and isinglass obtained from fish swim bladder. The degree of polymerization of polymeric proanthocyanidins that remained in the fined wine decreased significantly after addition of protein fining agents except when potassium caseinate was used. Furthermore, a study conducted by Maury et al. (2003) to examine the influence of protein fining on wines phenolic composition, showed that wheat glutens were selective in precipitating highly polymerized and galloylated tannin. Casein and isinglass induced a significant decrease in wine color (A420nm), a decrease in browning potential and a decrease in turbidity. Cosme et al. (2012) focused their research on determining if non-allergenic pea protein or polyvinylpolypyrrolidone (PVPP) are possible alternatives for casein fining. The results indicate that flavonoid and non-flavonoid phenols decreased in the wines treated with potassium caseinate, pea protein, and PVPP. All fining agents decreased wine color. Potassium caseinate was the most effective fining agent for reducing browning potential. When applying the CIELaB chromatic characterization, they found that the value for b* (yellowness) decreased significantly with all fining agents assayed; however, the decrease was greater in all experiments fined with potassium caseinate, indicating a higher reduction in the yellow intensity of the fined wine. Chroma (C*) is a parameter that indicates the contribution of a*(redness) and b*(yellowness). The value of C* decreased significantly after addition of pea protein, potassium caseinate and formulations of pea protein with PVPP. They found that PVPP could be used alone or in combination with much smaller quantities of casein and still effectively reduce wine oxidation through removal of polyphenols in reduced and oxidized (quinones and quinone methides) forms, which includes simple phenolic acids and flavonoids. Recently in 2013, Oberholster et al. investigated the effect of gelatin and egg albumin on the phenolic composition of Pinotage wine. They found that both gelatin and egg albumin fining decreased the mean degree of polymerization (mDP) of tannin significantly by

26.4% and 25.2%, respectively, compared to the control. Egg albumin treatments significantly decreased the total pigment content compared to control.

I.7. Conclusion

In this chapter, the chemistry of grape and wine polyphenols was revised as well as the biological activities of wine polyphenols. Also the impact of the winemaking techniques on wine polyphenols was reviewed and they are admitted to affect the phenolic composition of wines. Maceration, aging and clarification remain the most influencing steps while fermentation and filtration slightly impact the polyphenols content.

Despite all the progress made in this sector, some information remains contradictory. Moreover, the effects of some winemaking processes on wine polyphenols composition are still lacking. Therefore, more studies are required to elucidate the real impact of each step during the winemaking of a given wine.

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A – B – C

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Chapter II. Maceration Steps

PART 1- Terroir Effect

II.1.1. Introduction

Flavonoids and non-flavonoid compounds are both responsible for the sensorial characteristics of red wine and can exhibit antioxidant activities. These substances have a potentially positive effect on human health, thus giving to red wine "bioactive properties. Much has been published regarding the health benefits of wine (Auger et al., 2005; Femia et al., 2005; Basli et al., 2012; Hidalgo et al., 2012). These compounds act as potent antioxidants as they reduce low-density lipoprotein (LDL) cholesterol oxidation, modulate cell signaling pathways, reduce platelet aggregation, inhibit the growth of some tumor types, and exhibit anti-inflammatory, antibacterial, antifungal, antiviral, neuroprotective, anti-proliferative and anti-angiogenic activities. However, the beneficial effects of moderate wine consumption may be attributed to the overall mix of all of its components and not to a specific action of one.

The phenolic composition and content of red wine are affected by several factors, such as the grape (e.g., variety, ripening, cultivation, region) and winemaking techniques (e.g., maceration time and temperature, yeast and enzymes used, SO_2 dose, malolactic fermentation, clarification and filtration, ageing) (Andrades and Gonzàlez-Sanjosé, 1995; Ramos et al., 1999; Vrhovsek et al., 2002; Stankovic et al., 2012). Among these factors, maceration conditions have the largest impact on anthocyanins and tannins of the red wines, since these phenolic substances are mainly located in the skin, flesh and seed of the berries. For that reason different grape treatment methods have been applied to help the rupture of the cell structure of the berries in order to facilitate the release of phenolic compounds. The pre-fermentative maceration is defined as the period of time from filling into tanks with the crushed grapes to the beginning of the alcoholic fermentation. When it occurs at low temperature is called cold maceration or cold soak (usually carried out at temperatures between 10°C-15°C) and when it occurs at high temperature is called pre-fermentative mash heating or pre-fermentation heating maceration (usually carried out at temperatures between 65°C-80°C) with the target to improve some important quality characteristics of wines such as color and aroma (Netzel et al., 2003; Álvarez et al., 2006; Busse-Valverde et al., 2010). Temperature, skin contact time and wine growing regions are important factors to be considered in the results of the pre-fermentative macerations (Mateus et al., 2001; Vrhovsek et al., 2002; Orduña, 2010).

However, information about the evolution of phenolic compounds during the pre-fermentation heating maceration of red grapes varieties are scarce in the literature. Also, very little studies are available on Lebanese red wines and their phenolic composition. For that reason, the purpose of this work was first to determine the effect of maceration time and temperature on the chromatic characteristics, flavonoids and non-flavonoids profile and biological activities of Syrah and Cabernet Sauvignon musts elaborated in two distinct Lebanese wine growing regions, one located at West Bekaa (Thomas) and the other located at Chouf district (Florentine) using pre-fermentation cold and heat maceration compared to traditional winemaking scheme (control). Secondly, the objective was to elucidate by means of statistical multivariate analyses (PCA) the terroir effects as well as to define the best couple time/temperature of maceration for each grape must giving more information for a correct planning and management of the winemaking operations in the Lebanese terroir.

II.1.2. Materials and methods

II.1.2.1. CHEMICALS AND STANDARDS

All chemicals used were of analytical reagent grade. All chromatographic solvents (acetonitrile, acetic acid) were high-performance liquid chromatography (HPLC) grade and were purchased from Sigma-Aldrich (Steinheim, Germany). Delphinidin 3-O-glucoside, cyanidin 3-O-glucoside, peonidin-3-O-glucoside, malvidin 3-O-glucoside, (+) - Catechin, (-) – Epicatechin, (-) – Epicatechingallate (-) - Epigallocatechin, (-) - Epigallocatechingallate, Procyanidin B1, Procyanidin B2, Ferulic acid, Caffeic acid and trans-resveratrol were purchased from Extrasynthese (Genay, France). The Folin-Ciocalteu reagent, 1, 1-diphenyl- 2-picrylhydrazyl (DPPH) and 2, 2‟-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) were obtained from Sigma-Aldrich (Steinheim, Germany).

II.1.2.2. SAMPLES

Red grapes of *Vitis vinifera* var. Cabernet Sauvignon (CS) and Syrah (Sy) were supplied by two cellars from distinct regions: Clos St. Thomas (West Bekaa / Lebanon) and Chateau Florentine (Chouf District / Lebanon). Table II.1.1 resumes the soil type and regional climate conditions of each studied region. Grapes were harvested in 2014 at technological maturity (Brix= 21.2 and

23.2; titrable acidity = 4.4 and 3.7 g/l as sulfuric acid for Syrah St. Thomas and Syrah Florentine respectively, Brix= 24.2 and 26.4; titrable acidity = 3.7 and 3.1 g/l as sulfuric acid for CS Saint Thomas and CS Florentine respectively).

Table II.1.1: Wine producer, regional climate condition and soil type from the two different wine-growing regions.

Wine-growing region	Wine-producer	Soil type	Climate condition (2014 data)
West Bekaa/Lebanon	Clos St. Thomas	Limestone, pebbly clay, Clay-calcareous well drained, poor in humus and organic matter	The vineyards are located on the valley zones at the altitude of 950 m with a cool and semi-arid dry climate and a big difference between day and night time, with an annual rainfall of 650 mm, annual average temperature of 21.1°C.
Chouf District/Lebanon	Florentine	Clay-calcareous, stony basement	The vineyards are located on the mountains hills at the altitude of about 1000 m with a warm, dry sub-humid and temperate climate, with an annual rainfall of 1078 mm and annual average temperature of 15.1°C.

II.1.2.3. STRAINS AND STORAGE CONDITIONS

S. cerevisiae Y used in this work were kindly provided by Lallemand Inc. (Blagnac, France). Yeast stock cultures were kept at 4°C in YEPD (Yeast Extract Peptone Dextrose) agar slants composed of 10 g/l Yeast extract, 20 g/l peptone, 20 g/l D-glucose and 20 g/l agar. The yeast inoculum was prior prepared in two steps. First, a preculture of the yeast strain was obtained by reactivating the stock culture in YEPD broth for 24 h. Second, the preculture was used to inoculate a low sugar concentration synthetic grape juice medium composed of 50 g/l D-Glucose, 1 g/l Yeast extract, 2 g/l Ammonium sulfate, 0.3 g/l Citric acid, 5 g/l L-malic acid, 5 g/l L-tartaric acid, 0.4 g/l Magnesium sulfate and 5 g/l Potassium dihydrogen phosphate. This step was carried out for 48 h and provided the yeast inoculum.

II.1.2.4. MACERATION AND FERMENTATION PROCEDURES AND SAMPLING

After reception, the grapes were crushed and destemmed manually and sodium metabisulphite was added (5 g of $NaHSO_3$/100 kg). 2 kg lots of grapes were drawn into glass Erlenmeyer flasks of 2 L and the pre-fermentative macerations were conducted at different temperatures (10, 60, 70 and 80°C) for 48 hours. The macerations were monitored and the kinetic profile of the maceration was studied by taking samples at 0, 2, 4, 8, 24 and 48 hours. Classical winemaking process (maceration and fermentation occurs together at 25°C) of Syrah and Cabernet Sauvignon Saint Thomas were used as control. Musts issued from control were separately inoculated by *S. cerevisiae* Y yeast strain at an initial concentration of 3×10^6 cells/ml (Thoma counting chamber). The AF was followed until total or cessation of sugar consumption (< 2 g/l, DNS colorimetric method Miller, 1959) and finished after 10 days. Control samples were collected at the end of the alcoholic fermentation. At the latest 50 ml of each sample was collected and directly centrifuged for 5 minutes at 5000 rpm. The samples were stored at 0°C and analyses were done after the maceration and fermentation times (for the control) were finished. All macerations were carried out in triplicate.

II.1.2.5. SPECTROPHOTOMETRIC DETERMINATIONS

Chromatic parameters. The color density (CD) defined as the sum of absorbencies at 420 and 520 (Glories, 1984).

Total polyphenols index (TPI) was determined following the method described by Ribéreau Gayon et al. (1998). Wines were diluted with water (1:100) and the absorbance was measured directly at 280 nm.

Total anthocyanins were calculated by measurement of the absorbance at 520 nm after bisulfite bleaching solution. Total anthocyanin concentration was expressed in mg/l as described by (Ribereau-Gayon and Stonestreet, 1965).

Total tannins were determined by Bate-Smith method. Total tannins were determined by measurement of the absorbance at 550 nm after acid hydrolysis of the samples and a blank. Total tannins concentration was expressed in mg/l as described by (Ribereau-Gayon and Stonestreet, 1966).

Total phenolics were determined according to the Folin-Ciocalteu colorimetric method (Ribereau Gayon et al., 1972) and the results were expressed as gallic acid equivalent (mgGAE/l).

II.1.2.6. HPLC ANALYSIS OF PHENOLIC COMPOUNDS

The HPLC analyses were performed using a Shimadzu chromatographic system equipped with a quaternary pump (LC-20AD), an UV-Vis diode-array detector (SPD-M20A), an automatic injector (SIL-20A) and Shimadzu LC solution software. Samples (20µl injection volume) previously filtered through a 0.45µm cellulose acetate membrane (Greyhound Chromatography and Allied Chemicals, England), were injected on a Shim-pack VP-ODSC18 column (250*4.6 mm, 5µm particle size) protected with a guard column of the same material (10 mm x 4.6 mm, 5µm particle size) maintained at 40°C. All analyses were made in triplicate. The anthocyanin identification followed the method describing by Heredia et al. (2010) with some modifications, using acetonitrile/acetic acid/water (3:10:87, v/v/v) as solvent A and acetonitrile/acetic acid/water (50:10:40, v/v/v) as solvent B at a flow rate of 0.6 ml/min. The elution profile was as follows: 0-10 min 90% A-10% B; 10-13 min 85% A-15% B; 13-20 min 75% A-25% B; 20-40 min 45% A-55 % B; 40-43 min 100% B followed by washing and re-equilibration of the column. Quantification of flavan-3-ols and phenolic acids was performed following the method describing by Ducasse et al. (2010) with modifications. The elution conditions were as follows: 0.6 ml/min flow rate, solvent A, acetonitrile/acetic acid (97:3v/v); and solvent B acetic acid/water (3:97, v/v). The elution profile consists in 100% B for 0-25 min, 20% A-80% B for 25-45 min; 90% A-10% B for 45-55 min and then washing and re-equilibration of the column. Chromatograms were recorded at 520, 280 and 320 nm for anthocyanins, flavan-3-ols and phenolics acids respectively. Calibration curves were obtained for all phenols standards and the concentrations were expressed as mg/l.

II.1.2.7. DETERMINATION OF BIOLOGICAL ACTIVITIES

II.1.2.7.1. Preparation of samples

20 ml of musts were evaporated to dryness under vacuum using a rotary evaporator (35°C, 200 rpm). The must extracts were dissolved in dimethyl sulfoxide (DMSO) in order to obtain a final concentration of 50 mg/l in all microplate wells for antioxidant (ABTS and DPPH) assays and a

final concentration of 500 mg/l for anti-lipoxygenase (LOX, antiinflammatory), anti-cholinesterase (ChE, anti-Alzheimer), anti-xanthine oxidase (XOD), anti-α-glucosidase (antidiabetic) and cytotoxicity activities (anticancer). The total percentage of DMSO in the wells does not exceed 5%.

II.1.2.7.2. DPPH-radical scavenging assay (antioxidant activity)

Antioxidant scavenging activity was studied using 1, 1-diphenyl- 2-picrylhydrazyl free radical (DPPH) as described by Brand-Williams et al. (1995) with some modifications. DPPH was produced by mixing 7 mg of DPPH with 20 ml of methanol. The mixture was diluted with methanol in order to give absorbance measurements within the range of 0.6-0.8. 20 µl of the test materials (wine extracts) were mixed with 180 µl of a 0.8 mM methanolic DPPH solution. After an incubation period of 25 min at room temperature, the absorbance at 524 nm, the wavelength of maximum absorbance of DPPH, was recorded as A (sample), using UV/Vis microplate spectrophotometer (Multiskan^{TM} GO Thermo Scientific). A blank experiment was also carried out applying the same procedure to a solution without the test material and the absorbance was recorded as A (blank). The free radical-scavenging activity of each solution was then calculated as percent inhibition according to the following equation: %inhibition = 100(A (blank) – A (sample))/A (blank). Ascorbic acid was used as the standard. All measurements were performed in triplicate.

II.1.2.7.3. ABTS radical-scavenging assay (antioxidant activity)

The radical scavenging capacity of the samples for the ABTS (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) radical cation was determined as described by Re et al. (1999). ABTS was produced by mixing 7 mM of ABTS with 2.45 mM potassium persulfate ($K_2S_2O_8$) followed by storage in the dark at room temperature for 16 h before use. The mixture was diluted with water to give an absorbance measurements within the range of 0.7- 0.9 at 734 nm using a UV/Vis microplate spectrophotometer (Multiskan^{TM} GO Thermo Scientific). 20 µl for each sample was allowed to react with fresh ABTS solution (180 µl), and then the absorbance was measured 6 min after initial mixing. The radical-scavenging activity was expressed as percentage of inhibition and calculated in the same way as that previously used for the method of DPPH. Ascorbic acid was used as standard. All measurements were performed in triplicate.

II.1.2.7.4. LOX inhibition assay (anti-inflammatory activity)

Lipoxygenase (LOX) is an enzyme that catalyzes the oxidation of unsaturated fatty acids containing 1-4 diene structures. The conversion of linoleic acid to 13- hydroperoxy linoleic acid was followed spectrophotometrically by the appearance of a conjugate diene at 234 nm. (LOX) was assayed according to the method described by Axelrod et al. (1981), with some modifications. A mixture of a solution of phosphate buffer (150µl, 0.1 M, pH 7.4) and soybean LOX (10 µl, final conc. 8,000 U/ml) was incubated with must extract sample (20 µl) at 25°C for 10 min. The reaction was started by the addition of linoleic acid substrate (60 µl, 10 mmol). The absorbance of the resulting mixture was measured at 234 nm and recorded as A (sample) using an UV/Vis microplate reader (Multiskan^{TM} GO Thermo Scientific). A blank experiment was also carried out applying the same procedure to a solution without the test material and the absorbance was recorded as A (blank). Inhibition of LOX was calculated using the following equation: % of LOX inhibition = 100 x (A (blank) – A (sample))/A (blank). Nordihydroguaiaretic acid (NDGA) a known inhibitor of soybean lipoxygenase was used as positive control. All determinations were performed in triplicate.

II.1.2.7.5. Anti-XOD inhibition assay (anti-hyperuricemic effect)

Determination of Xanthine Oxidase (XOD) inhibitory activity was evaluated by measuring uric acid production from xanthine or hypoxanthine substrate at 295 nm as described by Kong et al. (2000), using a 96-well microplate reader (Multiskan^{TM} GO Thermo Scientific), with some modifications. The assay mixture consisted of 50 µl of sample solution, 60 µl (70mM) phosphate buffer (tampon, pH 7.5), 30 µl of enzyme solution (0.1 u/ml in buffer) and 60 µl of 150 µM xanthine. The reaction was initiated by the addition of the enzyme (XOD, incubated at 25°C for 15 min) afterwards the inhibition was evaluated by the addition of 60 µl of xanthine (incubated at 25°C for 5 min). Inhibition of XOD was calculated as following: % of XOD inhibition = 100 x (A (blank) – A (sample))/A (blank), where A (blank) is the absorbance of the control and A (sample) is the absorbance of the tested sample. Allopurinol was used as a positive control. All determinations were performed in triplicate.

II.1.2.7.6. Anti-ChE inhibition assay (anti-alzheimer activity)

Cholinesterase (ChE) inhibitory activities were measured using Ellman"s method (Ellman et al., 1961), with modifications. In this study, 50 µl of 0.1 M sodium phosphate buffer (pH 8.0), 25 µl of AChE solution, 25 µl of extract and 125 µl of DTNB were added in a 96-well microplate reader (Multiskan^{TM} GO Thermo Scientific), and incubated for 15 min at 25 °C. The reaction was then initiated with the addition of 25 µl of acetylthiocholine iodide (ActHi, incubated at 25°C for 10 min). The hydrolysis of acetylthiocholine iodide was monitored by the formation of the yellow 5-thio-2-nitrobenzoate anion as a result of the reaction of DTNB with thiocholine, catalyzed by enzymes at a wavelength of 412 nm. The percentage of inhibition was calculated as following: % of ChE inhibition = 100 x (A (blank) – A (sample))/A (blank), where A (blank) is the absorbance of the control and A (absorbance) is the absorbance of the test sample. Galanthamine hydrobromide (GaHBr) was used as positive control. All determinations were performed in triplicate.

II.1.2.7.7. α- Glucosidase inhibitory assay (antidiabetic activity)

The α-glucosidase inhibitory assay was referred to the method of Kim et al. (2008) with some modifications. Generally, the reaction mixture contained 25 µl of 0.1 M potassium phosphate buffer (pH 6.9), 25 µl of sample, and 50 µl of enzyme solution (1 U/ml). The reaction mixture was then incubated at 25 °C for 10 min. Then, the reaction was terminated by the addition of 25 µl of 5 mM 4-nitrophenyl-α-D-glucopyranoside (PNPG), incubated at 25 °C for 5 min. The increase in absorbance due to hydrolysis of PNPG by this enzyme was monitored at 405 nm on a UV/Vis microplate spectrophotometer (Multiskan^{TM} GO Thermo Scientific). The inhibition effect was calculated as follows: % α-glucosidase inhibition = ((absorbance of negative control − absorbance of sample)/absorbance of negative control) × 100. Acarbose was used as a standard inhibitor. All measurements were done in triplicate.

II.1.2.7.8. Cytotoxicity assay (anticancer activity)

Cytotoxicity of extracts was estimated on human breast cancer (MCF7) and human colon cancer (HCT116) as described by Natarajan et al. (2011) with modification. Cells were distributed in 96-well plates at $15*10^3$ cells / well in 100 µl of appropriate cell culture medium, and then 100 µl of extract were added, then the mixture was incubated at 37°C in a CO_2 incubator for 48

hours. Cell growth was estimated by the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay, based on the clivage of the tetrazolium salt by mitochondrial dehydrogenases in viable cells. The resulting blue formazan can be measured spectrophotometrically at 605 nm. The percentage of growth inhibition was calculated according to the following equation: % inhibition = ((absorbance of negative control − absorbance of sample)/absorbance of negative control) × 100. Tamoxifen was used as positive control. Each extract concentration was tested in triplicate.

II.1.2.8. STATISTICAL DATA TREATMENT

All experiments were carried out in triplicate. Analysis of variance (ANOVA) and Tukey‟s honestly significant difference (HSD) test were used for mean separation, with a significant level of 95% ($p < 0.05$).These statistical analyses, together with PCA, were conducted using Xlstat software (2014)

II.1.3. Results and discussion

II.1.3.1. IMPACT OF MACERATION'S TIME AND TEMPERATURE ON POLYPHENOL COMPOSITION OF MUSTS

II.1.3.1.1. Total anthocyanins and tannins

Figure II.1.1 and II.1.2 showed the evolution of total tannins versus total anthocyanins during the maceration of Syrah and Cabernet Sauvignon musts from the two distinct regions at different temperatures (10°C, 60°C, 70°C and 80°C). The results showed that temperature affects the amounts of tannins and anthocyanins released from skins and seeds to the must. By macerating at 10°C, the anthocyanin and tannin concentrations increases slightly during the 48 hours to reach a maximum of total anthocyanins of 129.79 mg/l; 140.29 mg/l; 193.37 mg/l and 223.12 mg/l respectively for Sy-ST, Sy-F, CS-ST and CS-F grape musts. The maximum concentration of tannins were ($[tanins]_{Sy\text{-}ST}$ = 3112.13 mg/l; $[tanins]_{Sy\text{-}F}$ = 2719.09 mg/l, $[tanins]_{CS\text{-}ST}$ = 5734.57 mg/l and $[tanins]_{CS\text{-}F}$ = 4639.20 mg/l). Contrariwise, temperature of 60°C showed a gradual increase in concentrations of total tannins and anthocyanins (compared to 10°C) for both grape varieties from the two different regions and reached it is maximum for tannins after 48 hours ($[tanins]_{Sy\text{-}ST}$ = 6301.58 mg/l, ($[tanins]_{Sy\text{-}F}$ = 7029.70 mg/l, ($[tanins]_{CS\text{-}ST}$ = 10038.71 mg/l, ($[tanins]_{CS\text{-}F}$ = 9375.05 mg/l) and for anthocyanins after 24 hours ($[anthocyanins]_{Sy\text{-}ST}$ = 633.79

mg/l, $[anthocyanins]_{Sy\text{-}F}$ = 822.79 mg/l, $[anthocyanins]_{CS\text{-}ST}$ =836.79 mg/l, $[anthocyanins]_{CS\text{-}F}$ =876.17 mg/l). A slight decrease is observed when the heating lasts up to 48 hours and concentrations reached for Syrah Saint Thomas, Syrah Florentine, Cabernet Sauvignon Saint Thomas and Cabernet Sauvignon Florentine were respectively 544.25 mg/l, 744.92 mg/l, 725.08 mg/l and 707.00 mg/l). For temperatures of 70°C and 80°C, the results showed an increase in concentrations of total anthocyanins and tannins compared to 10°C and 60°C. As for total anthocyanins, for Sy-ST grape must, concentrations reached 694.46 mg/l and 680.46 mg/l at 70°C and 80°C respectively after 4 hours. For Sy-F, the maximum concentration was 1033.08 mg/l at 70°C after 8 hours and 1005.60 mg/l at 80°C after 4 hours. For CS-ST and CS-F grape musts, concentration reached respectively 925.75 mg/l and 970.08 mg/l at 70°C after 8 hours and 802.08 mg/l and 951.42 mg/l at 80°C after 4 hours respectively for CS-ST and CS-F. Beyond these maximums, a significant decrease in total anthocyanins was observed for the different grape musts. This decrease is much greater for 80°C than for 70°C were concentrations were divided by an average factor of 5.64 and 2.26 for 80°C and 70°C respectively. Concerning tannins concentrations, at 70°C, and for the different grape musts, the maximum tannin extraction was achieved after 48h with a concentration of 8730.72 mg/l, 8833.81 mg/l, 11346.71 mg/l and 10579.95 mg/l for Sy-ST, Sy-F, CS-ST and CS-F respectively. For maceration at 80°C, the maximum extraction was reached faster after 24 hours of maceration with a value of 9806.75mg/l for CS-ST (except for CS-F when max concentration was reached after 8 hours). A slight decrease of 12.13%, 7.45%, 12.87% and 17.27% was noted respectively when maceration was prolonged for 48h for Sy-ST, Sy-F, CS-ST and CS-F. After alcoholic fermentation Sy-ST control (25°C), showed an anthocyanin concentration of 220.25 mg/l, this value being 1.70 and 1.80 times higher than Syrah Saint Thomas must macerated respectively at 10°C and 80°C after 48 hours, in addition anthocyanin concentration of CS-ST control (190.43 mg/l) was 1.39 times higher than Cabernet Sauvignon Saint Thomas macerated at 80°C after 48 hours. Besides, both control (Syrah and Cabernet Sauvignon Saint Thomas) showed lower tannin content than Thomas must macerated at different temperatures (data not shown).

This decrease in monomeric anthocyanins could be explained by the thermal degradation of anthocyanins at high temperatures and a shift in the equilibrium towards chalcone and colorless forms (Galvin 1993), oxidative cleavage of the heterocyclic ring leading to direct anthocyanin degradation (Morel -Salmi et al. 2006, Lopes et al. 2007) and the different reactions involving

anthocyanins during the extended maceration time (Gao et al., 1997; Gomez plaza et al., 2002). In opposition, longer maceration times seem to favor the extraction of tannins because the release of these compounds occurs from the grape skins and seeds. In the seeds flavan-3-ols are located in thin-walled cells between the external hydrophobic cuticle and the inner lignified layers so the release of these compounds from the seeds requires longer maceration times and high temperatures (Guerrero et al., 2009).

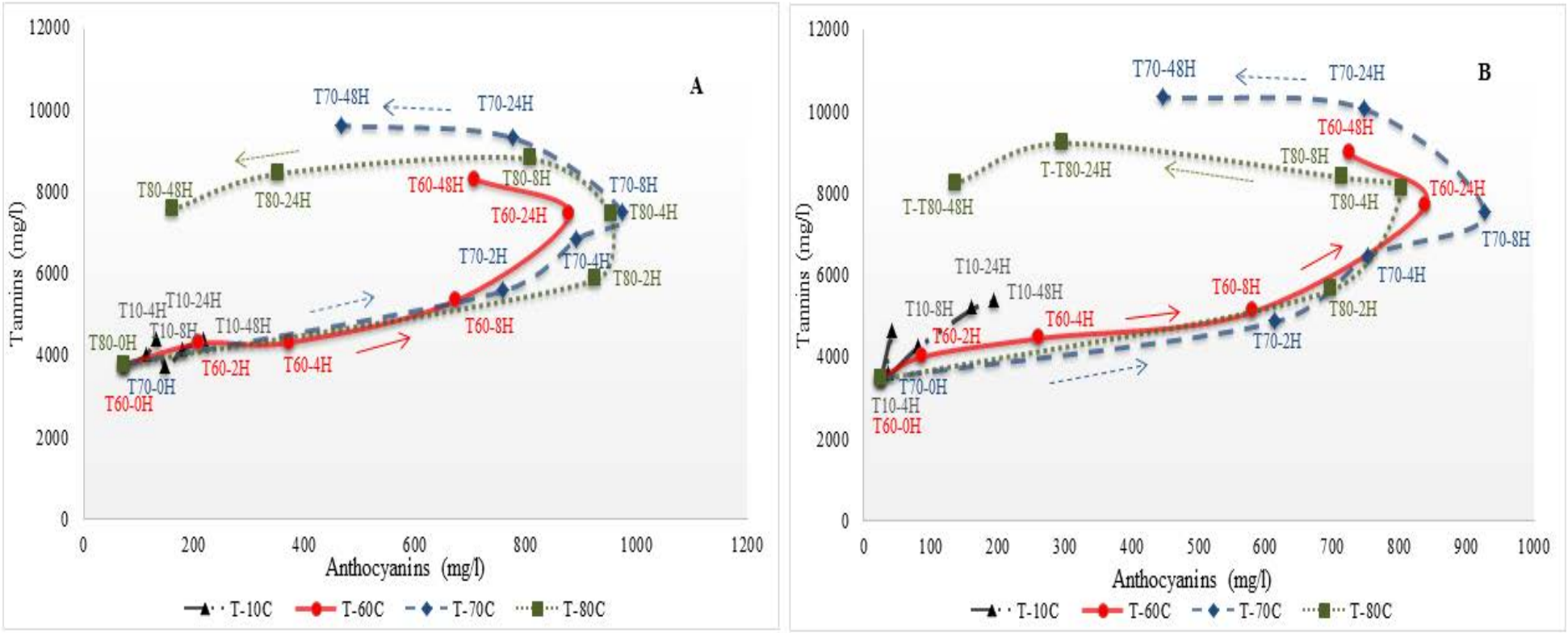

Figure II.1.1: Kinetics of tannins and anthocyanins extraction during the maceration of Cabernet Sauvignon grapes in terms of time and temperature (A: Chateau Florentine, B: Clos St Thomas, T-10C, T-60C, T-70C, T-80C: maceration temperatures respectively at 10°C, 60°C, 70°C and 80°C, example: T-60-4H: maceration temperature at 60°C for 4 hours)

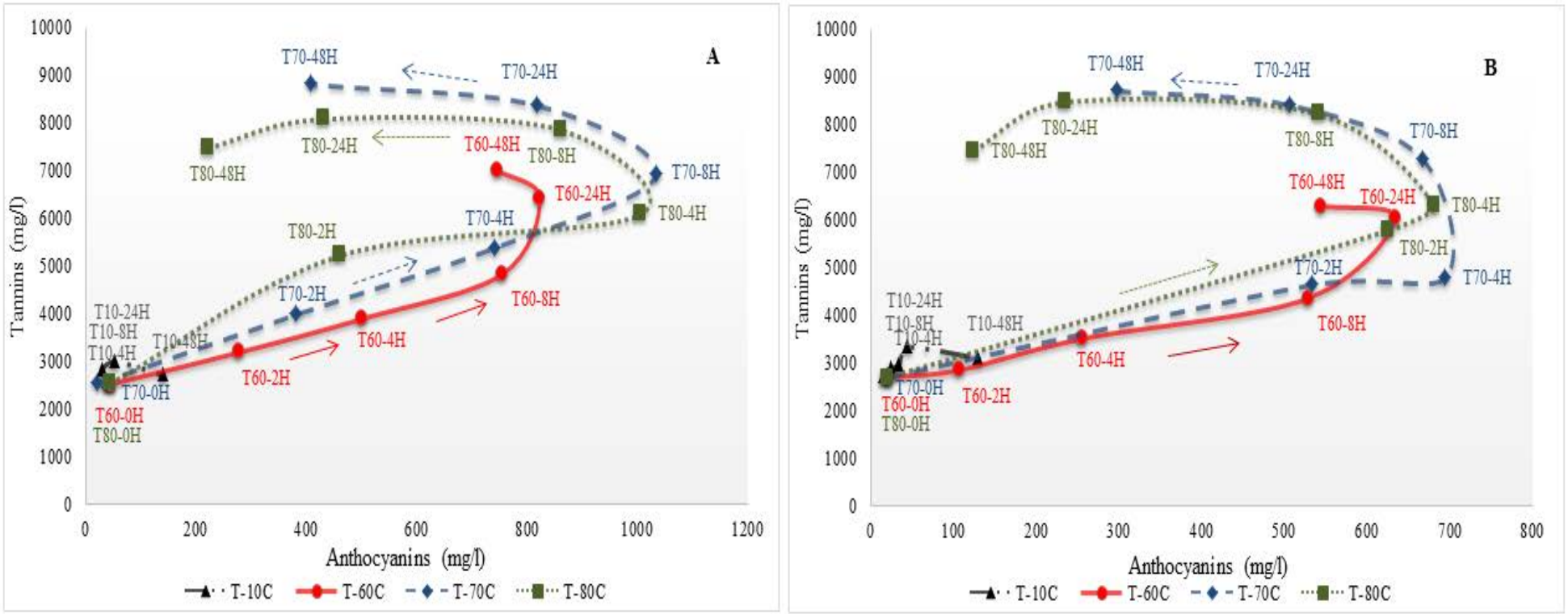

Figure II.1.2: Kinetics of tannins and anthocyanins extraction during the maceration of Syrah grapes in terms of time and temperature (A: Chateau Florentine, B: Clos St Thomas, T-10C, T-60C, T-70C, T-80C: maceration temperatures respectively at 10°C, 60°C, 70°C and 80°C, example: T-60-4H: maceration temperature at 60°C for 4 hours)

II.1.3.1.2. Total polyphenol, total polyphenol index and color intensity

Table II.1.2-a and II.1.2-b showed the evolution of total polyphenol, total polyphenol index and color intensity during the maceration of Syrah and Cabernet sauvignon musts from the two different regions at different temperatures (10°C, 60°C, 70°C, 80°C) compared to the control (classical winemaking at 25°C). Heating not only increased the total anthocyanins concentration, but also led to increase of color intensity. For the different grape musts, a slight increase in colour intensity was observed at the temperature of 10°C for 48 hours ($CI_{Sy\text{-}ST}$ = 0.55; $CI_{Sy\text{-}F}$ = 0.65; $CI_{CS\text{-}ST}$ = 0.54; $CI_{CS\text{-}F}$ = 0.68). A gradual increase was observed at 60°C, the color intensity reaching its maximum after 24 hours with a value of 1.53; 1.81; 1.46 and 2.19 for the Sy-ST, Sy-F, CS-ST and CS-F respectively. On the opposite, a high increase in color intensity was observed at 70°C this maximum was reached after 24 h for Sy-ST and CS-F ($CI_{Sy\text{-}ST}$ = 1.60; $CI_{CS\text{-}F}$ =2.08) and 8 h for Sy-F and CS-ST ($CI_{Sy\text{-}F}$ = 2.61; $CI_{CS\text{-}ST}$ =1.59). A significant increase in the color intensity up to 2 was observed after 48 hours for the Syrah Saint Thomas and Cabernet Sauvignon Florentine at 80°C. Therefore, Color intensity showed a similar tendency than that associated with anthocyanins (The higher values of CI corresponded to the higher values of anthocyanins) excepting for the temperature of 80°C for which the lower values of anthocyanins were associated with the higher values of CI. This can be explained by the formation of new compound due to copigmentation and condensations reactions (Galvin 1993). Florentine musts had the highest CI than Thomas musts after 48 hours of maceration. Moreover, the results showed an increase of total polyphenol index with temperature and maceration time (Table II.1.2-a; II.1.2-b). Low maceration temperature (10°C), did not allow any evolution of TPI over time. After 48 hours of maceration, the TPI values were 22.30; 62.30; 85.20 and 89.20 respectively at 10°C, 60°C, 70°C and 80°C respectively for Syrah Saint Thomas grape must, 18.07; 74.07; 88.20 and 86.80 at 10°C, 60°C and 70°C and 80°C respectively for Syrah Florentine grape must, 22.23; 61.33; 73.80 and 97.27 at 10°C, 60°C and 70 °C and 80°C respectively for Cabernet Sauvignon Saint Thomas grape must, 21.60; 64.43; 81.17 and 98.93 at 10°C, 60°C and 70°C and 80°C respectively for Cabernet Sauvignon Florentine grape must. The increase of phenolic compounds during maceration time (Table II.1.2-a; II.1.2-b) can be explained by the fact that the heat destroys the skins cell membranes, releasing the pigments, tannins and different phenolic substances into the must (Atanacković et al. 2012). In addition, a low presence of polyphenols was observed at 10°C due to an almost non-existent extraction.

Maceration of Syrah Saint Thomas produces a maximum of 683.33 mg/l (GAE), 606.67 mg/l (GAE) for Syrah Florentine, 886.67 mg/l (GAE) for Cabernet Sauvignon Saint Thomas and 840.00 mg/l (GAE) for Cabernet Sauvignon Florentine. At 60°C, an improved extraction of polyphenol was observed compared to that carried out at 10°C. The maximum extraction was reached at 48 h for the four musts. 2870 mg/l and 2846.67 mg/l (GAE) were the maximum concentration obtained for maceration of the grape musts of Cabernet Sauvignon. At 70°C, an increase of total polyphenols concentration was observed with a maximum at 48 hours. The maximum extraction was 4380 mg/l and 4660 mg/l (GAE) for Syrah Saint Thomas and Syrah Florentine respectively and 4380 mg/l and 4125.33 mg/l (GAE) for Cabernet Sauvignon Saint Thomas and Cabernet Sauvignon Florentine respectively. At 80°C, total polyphenols indicated faster rate of extraction with a maximum reached at 24 hours of 3730 mg/l (GAE) for Cabernet Sauvignon Saint Thomas. A maximum decrease of 17.2% in the total polyphenols was observed at 48 h. After alcoholic fermentation, both Thomas must controls showed higher values for color intensity, total polyphenol index and total polyphenols than musts macerated at 10°C (average values were 2.01; 2.62 and 3.21 times higher respectively for CI, TPI and TP) and lower values than that macerated at 60°C, 70°C and 80°C (average values were 1.35; 1.19 and 1.18 times lower respectively for CI, TPI and TP at 60°C, 1.32; 1.49; and 1.87 times lower respectively for CI, TPI and TP at 70°C and 1.80; 1.71 and 1.37 times lower respectively for CI, TPI and TP at 80°C) for the two different grape varieties and terroirs.

Table II.1.2-a: Total polyphenol, Total Polyphenol Index and Color Intensity of Syrah musts and Syrah Saint Thomas control in terms of time and temperature

		Sy maceration time (hours)												
			0		2		4		8		24		48	
		Control 25°C	F	ST	F	ST	F	ST	F	ST	F	ST	F	ST
10°C	CI	1.22 ± 0.01	0.42 ± 0.01^{a}	0.34 ± 0.01^{b}	0.43 ± 0.10^{a}	0.42 ± 0.21^{a}	0.44 ± 0.10^{a}	0.46 ± 0.01^{a}	0.49 ± 0.00^{a}	0.49 ± 0.00^{a}	0.59 ± 0.00^{a}	0.52 ± 0.00^{b}	0.65 ± 0.00^{a}	0.55 ± 0.00^{b}
	TPI	60.12 ± 2.57	16.20 ± 1.08^{a}	16.27 ± 0.50^{a}	16.22 ± 0.65^{b}	19.10 ± 0.70^{a}	16.27 ± 0.14^{b}	19.77 ± 0.78^{a}	16.30 ± 0.15^{b}	19.30 ± 0.96^{a}	15.27 ± 1.62^{b}	20.93 ± 0.94^{a}	18.07 ± 1.70^{b}	22.30 ± 0.87^{a}
	TP	2452.25 ± 46.19	628.33 ± 1.21^{a}	440.00 ± 0.50^{b}	605.00 ± 2.89^{a}	566.67 ± 0.40^{b}	583.00 ± 1.73^{a}	521.67 ± 0.81^{b}	555.00 ± 2.89^{a}	573.30 ± 6.07^{a}	540.00 ± 3.22^{b}	663.33 ± 3.09^{a}	606.67 ± 2.89^{b}	683.33 ± 0.20^{a}
60°C	CI	1.22 ± 0.01	0.42 ± 0.01^{a}	0.34 ± 0.00^{b}	0.80 ± 0.01^{a}	0.611 ± 0.03^{b}	1.20 ± 0.11^{a}	0.99 ± 0.02^{a}	1.67 ± 0.04^{a}	1.34 ± 0.10^{b}	1.81 ± 0.16^{a}	1.53 ± 0.01^{b}	1.80 ± 0.03^{a}	1.24 ± 0.09^{b}
	TPI	60.12 ± 2.57	16.93 ± 0.45^{a}	16.27 ± 0.25^{a}	26.80 ± 0.05^{a}	21.97 ± 0.50^{b}	38.30 ± 0.10^{a}	29.97 ± 2.90^{b}	52.15 ± 0.02^{a}	35.17 ± 2.80^{b}	64.53 ± 1.81^{a}	52.93 ± 1.62^{b}	74.07 ± 1.55^{a}	62.30 ± 0.63^{b}
	TP	2452.25 ± 46.19	628.33 ± 0.14^{a}	441.67 ± 0.81^{b}	927.20 ± 3.62^{a}	680.00 ± 3.41^{b}	1210.80 ± 0.10^{a}	873.30 ± 4.52^{b}	1648.90 ± 4.87^{a}	1393.33 ± 2.51^{b}	2490.00 ± 0.05^{a}	2266.67 ± 5.12^{a}	2756.70 ± 1.66^{a}	2643.30 ± 2.58^{a}
70°C	CI	1.22 ± 0.01	0.42 ± 0.00^{a}	0.34 ± 0.00^{b}	1.19 ± 0.11^{a}	1.30 ± 0.04^{a}	1.83 ± 0.02^{a}	1.39 ± 0.10^{b}	2.61 ± 0.00^{a}	1.59 ± 0.04^{b}	2.44 ± 0.09^{a}	1.60 ± 0.02^{b}	2.03 ± 0.01^{a}	1.30 ± 0.06^{b}
	TPI	60.12 ± 2.57	16.53 ± 0.40^{a}	16.70 ± 0.10^{a}	30.60 ± 0.35^{b}	37.43 ± 0.80^{a}	45.20 ± 0.15^{b}	49.93 ± 4.30^{a}	62.10 ± 0.46^{a}	56.00 ± 1.30^{b}	71.80 ± 1.14^{a}	73.73 ± 2.47^{a}	88.20 ± 1.80^{a}	85.20 ± 1.67^{a}
	TP	2452.25 ± 46.19	628.30 ± 3.63^{a}	440.00 ± 1.41^{b}	1296.60 ± 3.44^{b}	1526.67 ± 1.92^{a}	1878.40 ± 4.78^{b}	2155.00 ± 2.74^{a}	2656.60 ± 3.44^{b}	2758.33 ± 1.30^{a}	3185.00 ± 7.55^{b}	3585.00 ± 1.97^{a}	4660.00 ± 0.81^{a}	4380.00 ± 1.39^{b}
80°C	CI	1.22 ± 0.01	0.42 ± 0.01^{a}	0.34 ± 0.01	1.45 ± 0.62^{a}	1.47 ± 0.04^{a}	2.63 ± 0.01^{a}	1.52 ± 0.05^{b}	2.40 ± 0.00^{a}	1.66 ± 0.06^{b}	2.01 ± 0.17^{a}	1.93 ± 0.06^{a}	1.95 ± 0.02^{b}	2.031 ± 0.02^{a}
	TPI	60.12 ± 2.57	16.70 ± 0.11^{a}	16.37 ± 0.28	42.30 ± 0.11^{b}	45.47 ± 0.32^{a}	58.70 ± 0.11^{b}	60.87 ± 1.15^{a}	72.80 ± 0.40^{a}	73.17 ± 0.17^{a}	80.50 ± 0.29^{b}	85.80 ± 1.15^{a}	86.80 ± 0.00^{b}	89.20 ± 0.70^{a}
	TP	2452.25 ± 46.19	628.30 ± 4.20^{a}	440.00 ± 0.79	1852.00 ± 1.00^{b}	1875.00 ± 1.22^{a}	2732.60 ± 3.86^{b}	2823.33 ± 0.30^{a}	3108.70 ± 4.71^{b}	3301.67 ± 1.05^{a}	3542.80 ± 1.44^{b}	3661.67 ± 0.50^{a}	3329.60 ± 5.54^{a}	3031.67 ± 3.05^{b}

Mean (n =3) ± SD. For each maceration time from the two distinct regions, different letters in the same row indicate significant difference at $p < 0.05$. CI, Color intensity; TPI, total phenolic index; TP, total phenolic; Sy-ST, Syrah Saint Thomas; Sy-F, Syrah Florentine

Table II.1.2-b: Total polyphenol, Total Polyphenol Index and Color Intensity of Cabernet Sauvignon musts and Cabernet Sauvignon Saint Thomas control in terms of time and temperature

		CS maceration time (hours)												
			0		2		4		8		24		48	
		Control 25°C	F	ST	F	ST	F	ST	F	ST	F	ST	F	ST
10°C	CI	1.20 ± 0.01	0.14 ± 0.01^{a}	0.14 ± 0.00^{a}	0.29 ± 0.00^{a}	0.25 ± 0.00^{b}	0.35 ± 0.00^{a}	0.29 ± 0.00^{b}	0.43 ± 0.01^{a}	0.39 ± 0.01^{b}	0.55 ± 0.00^{a}	0.46 ± 0.00^{b}	0.68 ± 0.00^{a}	0.54 ± 0.00^{b}
	TPI	50.19 ± 0.04	15.60 ± 0.11^{a}	12.87 ± 0.40^{b}	21.83 ± 2.05^{a}	13.27 ± 0.60^{b}	16.70 ± 0.87^{b}	19.60 ± 1.25^{a}	17.57 ± 1.20^{a}	18.87 ± 1.00^{a}	17.50 ± 0.95^{a}	19.07 ± 1.66^{a}	21.60 ± 2.23^{a}	23.23 ± 1.72^{a}
	TP	2250.35 ± 5.77	680.00 ± 1.00^{a}	601.67 ± 2.58^{b}	691.67 ± 1.58^{a}	630.00 ± 1.71^{b}	656.67 ± 2.89^{a}	615.00 ± 3.22^{b}	685.00 ± 0.00^{b}	740.00 ± 1.00^{a}	726.67 ± 2.92^{b}	810.00 ± 1.41^{a}	840.00 ± 2.00^{b}	886.67 ± 0.63^{a}
60°C	CI	1.20 ± 0.01	0.07 ± 0.01^{b}	0.21 ± 0.01^{a}	0.50 ± 0.02^{a}	0.31 ± 0.01^{b}	0.71 ± 0.05^{a}	0.41 ± 0.03^{b}	1.03 ± 0.05^{a}	0.73 ± 0.04^{b}	2.19 ± 0.03^{a}	1.46 ± 0.02^{b}	2.11 ± 0.03^{a}	1.42 ± 0.02^{b}
	TPI	50.19 ± 0.04	15.47 ± 0.38^{a}	12.37 ± 0.20^{b}	17.70 ± 0.82^{a}	13.67 ± 0.50^{b}	18.23 ± 0.83^{a}	17.47± 0.15^{a}	28.03 ± 3.66^{a}	26.23 ± 1.13^{a}	49.53 ± 2.90^{a}	44.00 ± 1.00^{a}	64.43 ± 1.98^{a}	61.33 ± 0.15^{a}
	TP	2250.35 ± 5.77	680.00 ± 1.00^{a}	616.67 ± 2.20^{b}	858.33 ± 4.52^{a}	678.33 ± 2.67^{b}	1336.67 ± 1.52^{a}	815.00 ± 1.02^{b}	1590.00 ± 0.33^{a}	1350.00 ± 0.10^{a}	2201.67 ± 5.23^{a}	2160.00 ± 2.32^{a}	2846.67 ± 4.67^{a}	2870.00 ± 1.65^{a}
70°C	CI	1.20 ± 0.01	0.15 ± 0.01^{b}	0.20 ± 0.01^{a}	1.77 ± 0.01^{a}	0.70 ± 0.04^{b}	1.82 ± 0.05^{a}	1.017 ± 0.04^{b}	2.06 ± 0.03^{a}	1.59 ± 0.05^{b}	2.08 ± 0.05^{a}	1.49 ± 0.04^{b}	1.80 ± 0.01^{a}	1.28 ± 0.02^{b}
	TPI	50.19 ± 0.04	15.40 ± 0.30^{a}	12.77 ± 0.6^{b}	38.87 ± 3.20^{a}	27.30 ± 1.51^{b}	41.70 ± 3.18^{a}	31.40 ± 0.85^{b}	48.70± 0.36^{a}	45.63 ± 2.41^{a}	64.47 ± 1.47^{a}	62.73 ± 0.61^{a}	81.17 ± 3.55^{a}	73.80 ± 2.85^{a}
	TP	2250.35 ± 5.77	680.00 ± 2.00^{a}	601.67 ± 3.21^{b}	1678.33 ± 1.94^{a}	1325.00 ± 2.24^{a}	2560.00 ± 0.32^{a}	1745.00± 1.54^{b}	2665.00 ± 1.47^{a}	2520.00 ± 2.49^{a}	3711.67 ± 1.92^{a}	3766.67 ± 1.51^{a}	4125.33 ± 7.74^{a}	4380.00 ± 2.23^{a}
80°C	CI	1.20 ± 0.01	0.14 ± 0.00^{b}	0.20 ± 0.01^{a}	2.14 ± 0.05^{a}	0.93 ± 0.03^{b}	2.31 ± 0.02^{a}	1.20 ± 0.04^{b}	2.11 ± 0.02^{a}	1.36 ± 0.02^{b}	2.11 ± 0.01^{a}	1.79 ± 0.05^{a}	2.99 ± 0.03^{a}	1.74 ± 0.01^{b}
	TPI	50.19 ± 0.04	15.77 ± 0.65^{a}	12.63 ± 0.35^{b}	43.33 ± 2.95^{a}	35.73 ± 2.10^{b}	55.70 ± 2.75^{a}	41.83± 2.23^{b}	68.90 ± 2.74^{a}	54.80 ± 4.09^{b}	98.07 ± 1.20^{a}	88.77 ± 3.90^{b}	98.93 ± 0.75^{a}	97.27 ± 0.50^{a}
	TP	2250.35 ± 5.77	683.33 ± 2.88^{a}	600.00 ± 1.02^{b}	2420.00 ± 2.32^{a}	1973.33 ± 1.18^{b}	3311.67 ± 5.97^{a}	2668.30 ± 0.91^{b}	3525.00 ± 2.65^{a}	2990.00 ± 0.83^{b}	3588.33 ± 4.81^{a}	3730.00 ± 2.56^{a}	3280.00 ± 4.56^{a}	3221.67 ± 4.73^{a}

Mean (n =3) ± SD. For each maceration time from the two distinct regions, different letters in the same row indicate significant difference at $p < 0.05$.CI, Color intensity; TPI, total phenolic index; TP, total phenolic; CS-ST, Cabernet Sauvignon Saint Thomas; CS-F, Cabernet Sauvignon Florentine

II.1.3.1.3. Anthocyanins profile

The evolution of anthocyanins monomers during maceration of Syrah and Cabernet Sauvignon from the two different regions at different temperatures (10°C, 60°C, 70°C and 80°C) for 48 hours compared to the control (25°C) is shown in Tables (II.1.3-a, II.1.3-b). During the maceration of grape musts at 10°C, the presence of anthocyanins was almost zero. Malvidin-3-O-glucoside remains the most abundant compound found at this temperature with a maximum concentration of 20.7 mg/l for Cabernet Sauvignon from the two regions. Cyanidin-3-O-glucoside was no longer detected at this temperature. These results are in agreement with previous reports suggesting that malvidin 3-O-glucoside was found to be the main anthocyanin present in red grapes (De Nisco et al., 2013), furthermore, Cyanidin derivatives showed the lowest concentration probably because this anthocyanin is the precursor of all others (Núńez et al., 2004). Improved anthocyanin extraction was observed at 60°C compared to 10°C. The maximum extraction of Sy-ST must was reached at 24 h for delphinidin-3-O-glucoside (6.15 mg/l), cyanidin-3-O-glucoside (1.62 mg/l), peonidin-3-O-glucoside (10.97 mg/l) and malvidin-3-O-glucoside (85.39 mg/l), the maximum extraction of Sy-F must was reached at 48 hours for delphinidin-3-O-glucoside (12.25 mg/l) and cyanidin-3-O-glucoside (2.64 mg/l) and at 24 hours for peonidin-3-O-glucoside (10.66 mg/l) and malvidin-3-O-glucoside (77.92 mg/l). For Cabernet Sauvignon varieties from the two distinct regions, the maximum extraction at 60°C was reached at 24 h $[\text{delphinidin}]_{\text{CS-ST}}$ =11.74 mg/l; $[\text{delphinidin}]_{\text{CS-F}}$ =28.61mg/l); $[\text{cyanidin}]_{\text{CS-ST}}$ = 2.42 mg/l; $[\text{cyanidin}]_{\text{CS-F}}$ = 3.86 mg/l); $[\text{peonidin}]_{\text{CS-ST}}$ = 4.80 mg/l; $[\text{peonidin}]_{\text{CS-F}}$ = 8.39mg/l) and $[\text{malvidin}]_{\text{CS-ST}}$ =149.81 mg/l; $[\text{malvidin}]_{\text{CS-F}}$ = 174.44 mg/l). At 70°C, an increase in malvidin-3-O-glucoside with a maximum of 84.77 mg/l for Sy-ST was observed after 4 hours, 6 hours for Sy-F (153.89 mg/l) and an average of 153.5 mg/l respectively for CS-ST and CS-F after 8 hours. Following these peaks, a marked decrease reaching 7.52 mg/l, 9.16 mg/l and 20 mg/l was observed over time for Sy-ST, Sy-F and Cabernet Sauvignon from the two regions respectively. A faster decrease was detected for other anthocyanins at 48 hours. At 80°C, delphinidin-3-O-glucoside, cyanidin-3-O-glucoside and peonidin-3-O-glucoside, reached their maximum concentration after 4 h (6.26 mg/l, 1.70 mg/l and 9.56 mg/l respectively) while malvidin-3-O-glucoside peaked after 8 hours of maceration (81.71 mg/l). whereas for CS-ST, delphinidin-3-O-glucoside and cyanidin-3-O-glucoside reached their maximum concentration after 8 h (18.10 mg/l and 2.48 mg/l respectively), peonidin-3-O-glucoside and malvidin-3-O-glucoside (4.34 mg/l and 119.65 mg/l respectively) after 4 hours, while for CS-F, delphinidin-3-O-glucoside, cyanidin-3-O-glucoside and peonidin-3-O-glucoside reached their maximum concentration after 8 h (31.97 mg/l, 5.32 mg/l, 9.78mg/l

respectively) and malvidin-3-O-glucoside (157.36 mg/l) after 2 hours. Syrah and Cabernet sauvignon Saint Thomas controls showed higher values of delphinidin-3-O-glucoside, cyanidin-3-O-glucoside, peonidin-3-O-glucoside and malvidin-3-O-glucoside than all the other grape musts macerated at different temperatures (average values of Syrah control were 13.29; 1.14; 6.04 and 80.57 times higher than Syrah musts from the two regions after 48 h respectively at temperatures of 10°C, 60°C, 70°C and 80°C and average values were 4.92; 1.66; 1.93 and 75.81 times higher for CS control than CS musts from the two different regions after 48 h respectively at temperatures of 10°C, 60°C, 70°C and 80°C) (Table II.1.3-a; II.1.3-b). Eventually, monomeric anthocyanins detected by HPLC showed similar tendency than total anthocyanins analyzed by spectrophotometer.

The diffusion of anthocyanins during maceration is ensured by the breakdown of 2 biological barriers (cells walls and polysaccharides in the middle lamella). The diffusion is favored by the water-soluble nature of anthocyanins. Following the peak of anthocyanin extraction during maceration, a drop in concentrations is observed (Cheynier et al., 2006; Harberston et al., 2009). This loss of anthocyanins has been attributed to multiple factors such as: oxidative cleavage leading to anthocyanin degradation, copigmentation or reaction with other wine components, formation of pyranoanthocyanins and adsorption onto yeat cell walls and bitartrate crystals. As seen from our results and the literature, there is a negative relationship between maceration length and anthocyanins monomers concentration in the wines.

The influence of temperature on anthocyanins has been studied through thermal degradation of anthocyanins for blackberry (Wang and Xu, 2007), grape pomace (Mishra et al., 2008) and plums (Turturica et al., 2016). These studies showed that the thermal degradation of anthocyanins followed a first order reaction:

$$C_t = C_0 \exp(-kt) \quad (1)$$

Where C_t is anthocyanin concentration at time t of heating (min), C_0 is initial concentration of anthocyanins and K (min^{-1}) is the first order kinetic constant.

Estimation of the parameters for an isothermal process, such as kinetic parameters for anthocyanin degradation in juices and concentrates, is mathematically straightforward. Anthocyanins have been found to follow the 1st-order reaction kinetics and can be modeled using the Arrhenius relationship (Ahmed and others 2004):

$$k=k_{ref}\exp[-E_a/R.(1/T)] \quad (2)$$

k is the rate constant (min^{-1}), t is the heating time (min), k_{ref} is the frequency factor (min^{-1}), E is the activation energy (KJ/mole), R is the universal gas constant (8.314 J/mol.K) and T the absolute temperature (°K)

Table II.1.3-a: Anthocyanins profile (mg/l) of Syrah musts and Syrah Saint Thomas control in terms of time and temperature

		Sy maceration time (hours)												
			0		2		4		8		24		48	
		Control 25°C	F	ST	F	ST	F	ST	F	ST	F	ST	F	ST
10°C	Dp	6.00 ± 0.18	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
	Cy	3.12 ± 0.04	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
	Pn	6.10 ± 0.13	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	0.28 ± 0.01^{a}	0.17 ± 0.02^{b}	0.38 ± 0.01^{a}	0.27 ± 0.02^{b}
	MV	65.35 ± 0.51	1.538 ± 0.01^{a}	n.d	0.42 ± 0.02^{a}	0.33 ± 0.05^{b}	0.70 ± 0.00^{a}	0.351 ± 0.03^{b}	0.58 ± 0.01^{a}	0.36 ± 0.02^{b}	1.95 ± 0.02^{a}	1.83 ± 0.01^{b}	7.65 ± 0.03^{a}	2.78 ± 0.02^{b}
60°C	Dp	6.00 ± 0.18	n.d	n.d	0.89 ± 0.00^{a}	0.86 ± 0.01^{a}	2.82 ± 0.01^{a}	0.85 ± 0.00^{b}	6.84 ± 0.00^{a}	1.59 ± 0.19^{b}	8.53 ± 0.04^{a}	6.150 ± 0.19^{b}	12.25 ± 0.02^{a}	5.76 ± 0.17^{b}
	Cy	3.12 ± 0.04	n.d	n.d	n.d	n.d	1.57 ± 0.01^{a}	n.d	2.26 ± 0.03^{a}	1.36 ± 0.04^{b}	2.47 ± 0.04^{a}	1.62 ± 0.01^{b}	2.64 ± 0.04^{a}	1.42 ± 0.01^{b}
	Pn	6.10 ± 0.13	n.d	n.d	3.25 ± 0.02^{a}	0.98 ± 0.01^{b}	8.56 ± 0.03^{a}	1.85 ± 0.03^{b}	14.61 ± 0.01^{a}	6.06 ± 0.02^{b}	10.66 ± 0.02^{b}	10.97 ± 0.01^{a}	8.34 ± 0.00^{a}	6.53 ± 0.00^{b}
	MV	65.35 ± 0.51	1.53 ± 0.02^{a}	n.d	15.82 ± 0.01^{a}	9.79 ± 0.00^{b}	49.41 ± 0.00^{a}	15.16 ± 0.01^{b}	90.92 ± 0.01^{a}	40.56 ± 0.04^{b}	77.92 ± 0.03^{b}	85.39 ± 0.03^{a}	65.98 ± 0.02^{b}	53.67 ± 0.04^{b}
70°C	Dp	6.00 ± 0.18	n.d	n.d	8.12 ± 0.01^{a}	4.17 ± 0.01^{b}	18.77 ± 0.01^{a}	4.43 ± 0.04^{b}	16.26 ± 0.01^{a}	6.34 ± 0.02^{b}	9.16 ± 0.04^{a}	5.53 ± 0.00^{b}	4.54 ± 0.02^{a}	3.26 ± 0.03^{b}
	Cy	3.12 ± 0.04	n.d	n.d	0.98 ± 0.05^{b}	1.76 ± 0.00^{a}	5.94 ± 0.02^{a}	1.84 ± 0.02^{b}	3.55 ± 0.01^{a}	1.56 ± 0.03^{b}	2.49 ± 0.03^{a}	1.37 ± 0.01^{b}	1.10 ± 0.01^{a}	n.d
	Pn	6.10 ± 0.13	n.d	n.d	14.89 ± 0.00^{a}	9.44 ± 0.03^{b}	28.16 ± 0.01^{a}	10.46 ± 0.03^{b}	18.74 ± 0.02^{a}	7.16 ± 0.03^{b}	6.87 ± 0.01^{a}	4.77 ± 0.03^{b}	1.13 ± 0.03^{a}	0.69 ± 0.04^{b}
	MV	65.35 ± 0.51	1.52 ± 0.02^{a}	n.d	65.81 ± 0.01^{a}	56.14 ± 0.04^{b}	153.89 ± 0.00^{a}	84.77 ± 0.03^{b}	139.92 ± 0.01^{a}	42.05 ± 0.02^{b}	37.76 ± 0.05^{a}	28.73 ± 0.00^{b}	9.16 ± 0.21^{a}	7.52 ± 0.02^{b}
80°C	Dp	6.00 ± 0.18	n.d	n.d	8.36 ± 0.01^{a}	5.56 ± 0.02^{b}	19.32 ± 0.01^{a}	6.26 ± 0.04^{b}	15.30 ± 0.00^{a}	5.85 ± 0.04^{b}	n.d	n.d	n.d	n.d
	Cy	3.12 ± 0.04	n.d	n.d	2.30 ± 0.20^{a}	2.01 ± 0.04^{a}	3.80 ± 0.00^{a}	1.70 ± 0.03^{b}	1.47 ± 0.01^{a}	1.42 ± 0.01^{a}	0.60 ± 0.00^{a}	n.d	n.d	n.d
	Pn	6.10 ± 0.13	n.d	n.d	18.90 ± 0.00^{a}	10.23 ± 0.02^{b}	22.30 ± 0.00^{a}	9.56 ± 0.01^{b}	5.40 ± 0.00^{a}	5.46 ± 0.03^{a}	0.80 ± 0.00^{a}	0.15 ± 0.04^{b}	n.d	n.d
	MV	65.35 ± 0.51	1.35 ± 0.01^{a}	n.d	77.80 ± 0.00^{a}	62.85 ± 0.03^{b}	158.70 ± 0.41^{a}	77.96 ± 0.03^{b}	132.50 ± 0.00^{a}	81.74 ± 0.01^{b}	8.96 ± 0.00^{a}	3.42 ± 0.01^{b}	n.d	n.d

Mean (n =3) ± SD. For each maceration time from the two distinct regions, different letters in the same row indicate significant difference at $p < 0.05$. Dp, delphinidin-3-O-glucoside; Cy, cyanidin-3-O-glucoside; Pn, peonidin-3-O-glucoside; Mv, malvidin-3-O-glucoside; Sy-ST, Syrah Saint Thomas; Sy-F, Syrah Florentine; n.d., not detected values

Table II.1.3-b: Anthocyanins profile (mg/l) of Cabernet Sauvignon musts and Cabernet Sauvignon Saint Thomas control in terms of time and temperature

		CS maceration time (hours)												
			0		2		4		8		24		48	
		Control 25°C	F	ST	F	ST	F	ST	F	ST	F	ST	F	ST
10°C	Dp	4.63 ± 0.30	n.d	n.d	0.89 ± 0.01^{a}	n.d	1.32 ± 0.01^{a}	n.d	0.93 ± 0.03^{a}	n.d	0.95 ± 0.01^{a}	0.87 ± 0.013^{b}	0.98 ± 0.00^{a}	0.84 ± 0.01^{b}
	Cy	1.91 ± 0.00	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
	Pn	2.92 ± 0.01	0.13 ± 0.00^{a}	n.d	0.16 ± 0.00^{a}	n.d	0.29 ± 0.00^{a}	n.d	0.32 ± 0.00^{a}	0.22 ± 0.02^{b}	0.29 ± 0.01^{a}	0.11 ± 0.00^{a}	0.39 ± 0.01^{a}	0.24 ± 0.01^{b}
	MV	66.35 ± 1.98	4.89 ± 0.05^{a}	1.14 ± 0.02^{b}	8.47 ± 0.01^{a}	4.24 ± 0.04^{b}	13.44 ± 0.03^{a}	2.30 ± 0.04^{b}	14.26 ± 0.01^{a}	10.2 ± 0.03^{b}	19.2 ± 0.05^{b}	20.65 ± 0.11^{a}	13.8 ± 0.01^{b}	14.57 ± 0.03^{a}
60°C	Dp	4.63 ± 0.30	n.d	n.d	0.93 ± 0.03^{a}	n.d	1.26 ± 0.05^{a}	0.8 ± 0.04^{b}	7.00 ± 0.04^{a}	4.24 ± 0.04^{b}	28.61 ± 0.03^{a}	11.74 ± 0.03^{b}	12.38 ± 0.01^{a}	6.46 ± 0.05^{b}
	Cy	1.91 ± 0.00	n.d	n.d	0.00 ± 0.00^{a}	n.d	1.18 ± 0.02^{a}	0.00 ± 0.00^{b}	1.59 ± 0.03^{a}	1.10 ± 0.02^{b}	3.86 ± 0.02^{a}	2.42 ± 0.02^{b}	1.54 ± 0.03^{a}	1.25 ± 0.04^{b}
	Pn	2.92 ± 0.01	0.12 ± 0.00^{a}	n.d	0.77 ± 0.01^{a}	0.08 ± 0.02^{b}	3.26 ± 0.04^{a}	0.65 ± 0.05^{b}	6.61 ± 0.01^{a}	3.20 ± 0.032^{b}	8.39 ± 0.03^{a}	4.8 ± 0.04^{b}	3.98 ± 0.01^{a}	1.73 ± 0.05^{b}
	MV	66.35 ± 1.98	5.79 ± 0.01^{a}	1.20 ± 0.05^{b}	17.33 ± 0.02^{a}	16.25 ± 0.04^{b}	48.85 ± 0.05^{a}	29.04 ± 0.05^{b}	112.91 ± 0.02^{a}	85.17 ± 0.05^{b}	174.44 ± 0.02^{a}	149.81 ± 0.02^{b}	57.03 ± 0.05^{a}	47.83 ± 0.05^{b}
70°C	Dp	4.63 ± 0.30	n.d	n.d	8.94 ± 0.04^{a}	4.24 ± 0.01^{b}	24.98 ± 0.05^{a}	7.36 ± 0.01^{b}	33.14 ± 0.05^{a}	14.33 ± 0.02^{b}	29.22 ± 0.05^{a}	18.26 ± 0.03^{b}	18.86 ± 0.05^{a}	12.38 ± 0.01^{b}
	Cy	1.91 ± 0.00	n.d	n.d	2.53 ± 0.05^{a}	1.23 ± 0.01^{b}	3.86 ± 0.01^{a}	1.62 ± 0.1^{b}	4.35 ± 0.05^{a}	2.10 ± 0.05^{b}	1.98 ± 0.05^{a}	1.54 ± 0.02^{b}	1.78 ± 0.01^{a}	1.32 ± 0.03^{b}
	Pn	2.92 ± 0.01	n.d	n.d	6.90 ± 0.07^{a}	3.41 ± 0.01^{b}	9.65 ± 0.09^{a}	4.29 ± 0.02^{b}	11.43 ± 0.03^{a}	5.70 ± 0.03^{b}	5.83 ± 0.01^{a}	3.59 ± 0.05^{b}	0.84 ± 0.01^{a}	0.28 ± 0.01^{b}
	MV	66.35 ± 1.98	4.47 ± 0.02^{a}	1.24 ± 0.05^{b}	88.57 ± 0.02^{a}	73.63 ± 0.03^{b}	136.90 ± 0.02^{a}	121.90 ± 0.01^{b}	156.66 ± 0.02^{a}	151.01 ± 0.02^{a}	105.46 ± 0.03^{a}	82.32 ± 0.05^{b}	23.39 ± 0.05^{a}	20.79 ± 0.04^{b}
80°C	Dp	4.63 ± 0.30	n.d	0.00 ± 0.00^{a}	17.33 ± 0.04^{a}	9.36 ± 0.02^{b}	31.97 ± 0.03^{a}	13.38 ± 0.01^{b}	20.42 ± 0.32^{a}	18.10 ± 0.05^{b}	8.82 ± 0.32^{a}	8.12 ± 0.03^{a}	n.d	0.00 ± 0.00^{a}
	Cy	1.91 ± 0.00	n.d	0.00 ± 0.00^{a}	2.72 ± 0.04^{a}	1.70 ± 0.05^{b}	5.32 ± 0.01^{a}	2.12 ± 0.03^{b}	5.15 ± 0.04^{a}	2.48 ± 0.05^{b}	1.07 ± 0.00^{b}	1.09 ± 0.01^{a}	n.d	0.00 ± 0.00^{a}
	Pn	2.92 ± 0.01	0.14 ± 0.01^{a}	0.00 ± 0.00^{b}	6.47 ± 0.07^{a}	3.89 ± 0.03^{b}	9.78 ± 0.23^{a}	4.34 ± 0.04^{b}	4.62 ± 0.09^{a}	3.62 ± 0.02^{b}	0.27 ± 0.00^{a}	0.17 ± 0.01^{b}	n.d	0.00 ± 0.00^{a}
	MV	66.35 ± 1.98	9.64 ± 0.09^{a}	1.24 ± 0.01^{b}	157.36 ± 2.91^{a}	91.47 ± 0.02^{b}	142.69 ± 0.05^{a}	119.65 ± 0.03^{b}	71.75 ± 0.05^{b}	81.75 ± 0.05^{a}	9.25 ± 0.03^{a}	7.27 ± 0.05^{b}	n.d	0.410 ± 0.01^{a}

Mean (n =3) ± SD. For each maceration time from the two distinct regions, different letters in the same row indicate significant difference at $p < 0.05$. Dp, delphinidin-3-O-glucoside; Cy, cyanidin-3-O-glucoside; Pn, peonidin-3-O-glucoside; Mv, malvidin-3-O-glucoside; CS-ST, Cabernet Sauvignon Saint Thomas; CS-F, Cabernet Sauvignon Florentine; n.d., not detected values.

II.1.3.1.4. Flavan-3-ols and non-flavonoids profile

The flavan-3-ol monomers, proanthocyanidins dimers, phenolic acids and stilbenes were identified and quantified (Table II.1.4-a, II.1.4-b) during maceration of the two grape varieties from the two different regions at different temperatures (10°C, 60°C, 70°C and 80°C) for 48 hours compared to the control (Sy and CS-ST-25°C). Regarding momomeric tannins the extraction of catechin, epicatechin, epicatechin gallate and epigallocatechin was favored by high temperatures compared to the low temperature (10°C) and even when treatment is prolonged over time for the different grape musts. The concentration of these monomers gradually increases with increasing temperature to maximum values of 97.12 mg/l for CS-ST-70°C after 24 hours, 158.30 mg/l for Sy-F-80°C after 48 hours, 156.15 mg/l for CS-ST-80°C after 48 hours and 984.73 mg/l for CS-ST-80°C after 24 hours respectively for catechin, epicatechin, epicatechin gallate and epigallocatechin. As for dimeric tannins, the low temperature (10°C) does not promote extraction of procyanidin B1 and B2. Higher extraction was favoured by high temperatures. At 60°C, a remarkable increase was observed compared to 10°C. The maximum extraction at this temperature was reached at 48 hours for the different musts (except for Cabernet Sauvignon musts, where the maximum concentration of pro B1 was reached after 24 hours). A very marked increase in procyanidins was observed at 70°C with a maximum reached at 24 hours for CS-ST ([pro B1] = 279.59 mg/l) and a maximum at 48 hours for CS-F ([pro B2] = 372.14 mg/l). For maceration at 80°C, the maximum concentration of pro B1 peaked for CS-ST at 24 hours and the maximum procyanidin B2 was achieved at 48 hours for CS-F, and then a significant loss was observed beyond that time. In addition, concerning the hydroxybenzoic acids, low concentrations of gallic acid were noted when macerating at 10°C for all must grapes. The evolution over time is almost nonexistent. The extraction of gallic acid was favored by high temperatures around 8 hours for Syrah Florentine and Cabernet Sauvignon Saint Thomas. At 48 h for 60°C, 70°C and 80°C gallic acid is no longer detected by liquid chromatography which means that this compound is very sensitive to heat and degraded completely over time. Cabernet Sauvignon Saint Thomas had the maximum concentrations of 13.87 mg/l after 8 h of maceration at 80°C. Unlike gallic acid, caffeic and ferulic acid were not very sensitive to high temperatures. The results obtained from Table II.1.4-a; II.1.4-b showed that heat promotes caffeic and ferulic acid extraction compared to low temperature (10°C). The maximum extraction was obtained for 48 hours at 60°C, 70°C and 80°C. Syrah Florentine showed the max concentration of ferulic acid (48.40 mg/l) after 48 hours at 70°C and caffeic acid (24.80 mg/l) after 48 hours at 80°C. After all, The extraction of resveratrol increased progressively as temperature increases and during the time to reach a concentration

2.37 times higher for Sy- F (50.90 mg/l, 70°C, 48h) than Sy-ST (21.47 mg/l, 80°C, 48h) and 1.20 times higher for CS-F (53.33 mg/l, 60°C, 48h) than CS-ST (44.38 mg/l, 70°C, 48h). It was observed in previously published results (Romero-Perez et al., 2001) that the maximum extraction for total resveratrol occurs at 60°C for 30 min, and that a higher increase in temperature is not related to a higher increase in the extraction. These results are inconsistent with our presented findings, since in the most cases an increase of temperature resulted in the enhancement of resveratrol content. By comparing the results obtained to the control, Table II.1.4-a and II.1.4-b showed that Sy-ST control exhibited higher values of phenolic acids compared to Syrah musts macerated at different temperatures after 48 hours, whereas CS-ST control showed higher values of gallic acid compared to Cabernet Sauvignon musts macerated at different temperatures. Ultimately, With the exception of gallic acid, which showed a high temperature-sensitive, all the tannins revealed an increase in concentration with temperature and macerating time, which coincides with the values of total polyphenols obtained by spectrophotometric determinations.

In the musts and wines of *V. vinifera* grapes, flavan-3-ols appear as 4 monomeric units: (+)-catechin, (-)-epicatechin, (+)-epigallocatechin and (-) epicatechin-3-O-gallate distributed diiferently within the berry tissues. Seeds contain (+)-catechin, (-)-epicatechin and (-) epicatechin-3-O-gallate (Prieur et al., 1994) whereas skins additionally contain (-)-epigallocatechin. Our results showed that (-)-epigallocatechin was extracted rapidly and in higher concentration that the other monomers which indicates that the skin tannins are extracted preferentially during the first hours of maceration. These results are according with those obtained by Gonzalez-Monzano et al., (2004) and Guerrero et al., (2009) showed that the release of flavan-3-ols from the seeds requires longer maceration times. The time needed in other studies to obtain high concentration in tannins (Gonzalez-Monzano et al., 2004; Hernanadez-Jimenez et al., 2012) are higher than those obtained in this study because high temperatures weaken the cells which accelerate the diffusion and the extraction of tannins.

For oligomers (Procyanidin B1 and B2), studies showed that skins dimeric proanthocyanidins are preferentially extracted during the early stages of maceration (Koyama et al., 2007).The diffusion of dimers follows extraction kinetics to those reported for skin‟s flavan-3-ols.

Table II.1.4-a: Flavan-3-ols and non-flavonoids profile (mg/l) of Syrah musts and Syrah Saint Thomas control in terms of time and temperature

		Sy maceration time (hours)													
			0		2		4		8		24		48		
		Control 25°C	F	ST	F	ST	F	ST	F	ST	F	ST	F	ST	
10°C	Cat	53.00 ± 0.34	13.64 ± 0.13^{a}	11.63 ± 0.38^{b}	13.92 ± 0.00^{a}	10.53 ± 0.04^{b}	15.49 ± 0.00^{a}	6.55 ± 0.21^{b}	20.19 ± 0.00^{a}	12.48 ± 0.11^{b}	12.67 ± 0.25^{a}	7.30 ± 0.12^{b}	17.46 ± 0.21^{a}	14.51 ± 0.51^{b}	
	Epi	90.22 ± 0.76	3.05 ± 0.05^{a}	2.18 ± 0.07^{b}	3.65 ± 0.01^{a}	3.71 ± 0.06^{a}	3.87 ± 0.02^{b}	4.96 ± 0.02^{a}	6.76 ± 0.05^{a}	5.29 ± 0.02^{b}	9.67 ± 0.28^{a}	7.58 ± 0.23^{b}	10.43 ± 0.41^{a}	8.00 ± 0.30^{b}	
	Epig	22.13 ± 0.89	1.91 ± 0.08^{a}	1.98 ± 0.00^{a}	2.86 ± 0.02^{b}	3.28 ± 0.05^{a}	3.73 ± 0.52^{a}	2.50 ± 0.03^{b}	6.31 ± 0.52^{a}	5.02 ± 0.18^{b}	10.45 ± 0.39^{a}	6.64 ± 0.11^{b}	7.15 ± 0.21^{a}	7.18 ± 0.30^{a}	
	EpiG	72.32 ± 0.29	0.00 ± 0.00^{b}	37.77 ± 0.59^{a}	35.65 ± 0.23^{b}	55.48 ± 0.07^{a}	38.90 ± 0.02^{b}	42.19 ± 0.92^{a}	41.90 ± 0.09^{b}	80.31 ± 1.54^{a}	44.89 ± 0.58^{b}	98.01 ± 4.88^{a}	56.89 ± 0.07^{b}	102.04 ± 4.7^{a}	
	Pro B1	110.05 ± 0.28	2.76 ± 0.08^{b}	4.47 ± 0.02^{a}	3.82 ± 0.13^{b}	5.49 ± 0.07^{a}	5.83 ± 0.11^{b}	8.29 ± 0.19^{a}	6.09 ± 0.05^{b}	7.31 ± 0.18^{a}	8.21 ± 0.18^{a}	8.34 ± 0.47^{a}	8.79 ± 0.14^{a}	8.58 ± 0.07^{a}	
	Pro B2	115.32 ± 0.32	16.45 ± 0.28^{a}	7.38 ± 0.28^{b}	17.24 ± 0.01^{a}	10.79 ± 0.13^{b}	18.71 ± 0.00^{a}	11.29 ± 0.20^{b}	26.04 ± 0.01^{a}	22.01 ± 0.45^{b}	32.59 ± 1.43^{a}	24.39 ± 0.32^{b}	40.26 ± 0.13^{a}	26.47 ± 0.64^{b}	
	G.A	25.10 ± 0.10	1.89 ± 0.03^{a}	1.93 ± 0.01^{a}	1.90 ± 0.12^{a}	1.98 ± 0.03^{a}	1.91 ± 0.04^{b}	2.13 ± 0.03^{a}	1.98 ± 0.14^{a}	2.07 ± 0.02^{a}	1.88 ± 0.03^{b}	2.02 ± 0.04^{a}	1.90 ± 0.01^{a}	1.98 ± 0.08^{a}	
	F.A	60.22 ± 0.40	1.82 ± 0.03^{b}	1.92 ± 0.01^{a}	1.88 ± 0.03^{b}	2.41 ± 0.03^{a}	1.95 ± 0.02^{b}	2.32 ± 0.06^{a}	2.69 ± 0.18^{b}	3.16 ± 0.10^{a}	3.72 ± 0.05^{b}	5.68 ± 0.25^{a}	4.76 ± 0.08^{b}	6.63 ± 0.12^{a}	
	C.A	25.08 ± 0.15	1.86 ± 0.02^{b}	1.95 ± 0.02^{a}	1.92 ± 0.00^{b}	2.13 ± 0.04^{a}	1.95 ± 0.03^{b}	2.22 ± 0.05^{a}	2.27 ± 0.03^{a}	2.38 ± 0.03^{a}	2.43 ± 0.01^{a}	2.47 ± 0.10^{a}	2.42 ± 0.08^{a}	2.07 ± 0.02^{b}	
	Res	7.14 ± 0.00	1.45 ± 0.00^{a}	1.48 ± 0.02^{a}	1.49 ± 0.02^{a}	1.49 ± 0.00^{a}	1.51 ± 0.12^{a}	1.46 ± 0.02^{a}	1.47 ± 0.02^{a}	1.55 ± 0.03^{a}	2.29 ± 0.09^{a}	1.73 ± 0.04^{b}	2.80 ± 0.04^{b}	3.61 ± 0.17^{a}	
60°C	Cat	53.00 ± 0.34	13.70 ± 0.43^{a}	8.68 ± 0.17^{b}	19.74 ± 0.01^{a}	12.09 ± 0.26^{b}	33.68 ± 0.01^{a}	9.54 ± 0.09^{b}	28.93 ± 0.00^{a}	18.81 ± 0.64^{b}	32.62 ± 0.23^{a}	32.21 ± 0.31^{b}	34.29 ± 0.70^{a}	36.43 ± 1.41^{a}	
	Epi	90.22 ± 0.76	3.63 ± 0.12^{a}	2.07 ± 0.07^{b}	8.63 ± 0.00^{a}	7.48 ± 0.08^{b}	11.49 ± 0.10^{a}	7.87 ± 0.27^{b}	28.95 ± 0.35^{a}	21.45 ± 0.51^{b}	57.83 ± 2.11^{a}	41.32 ± 1.20^{b}	80.71 ± 0.31^{a}	71.18 ± 1.65^{b}	
	Epig	22.13 ± 0.89	2.75 ± 0.10^{a}	1.95 ± 0.02^{b}	7.65 ± 0.65^{a}	5.33 ± 0.02^{b}	14.73 ± 0.45^{a}	10.07 ± 0.24^{b}	17.45 ± 0.02^{a}	14.79 ± 0.32^{b}	44.89 ± 2.00^{a}	12.35 ± 0.39^{b}	46.72 ± 0.88^{a}	15.64 ± 0.33^{b}	
	EpiG	72.32 ± 0.29	n.d	33.40 ± 0.15^{a}	79.50 ± 0.91^{a}	76.61 ± 0.12^{a}	113.84 ± 0.50^{a}	62.76 ± 0.14^{b}	274.69 ± 0.06^{a}	209.02 ± 0.41^{b}	296.23 ± 3.08^{a}	216.95 ± 0.41^{b}	523.33 ± 4.25^{a}	283.68 ± 1.10^{b}	
	Pro B1	110.05 ± 0.28	2.73 ± 0.04^{b}	4.39 ± 0.06^{a}	12.30 ± 0.03^{a}	7.26 ± 0.24^{b}	46.19 ± 0.01^{a}	16.37 ± 0.50^{b}	88.60 ± 0.00^{a}	43.51 ± 1.38^{b}	115.97 ± 2.86^{b}	225.89 ± 0.53^{a}	194.91 ± 0.84^{b}	265.65 ± 0.82^{a}	
	Pro B2	115.32 ± 0.32	9.27 ± 0.21^{a}	6.34 ± 0.23^{b}	13.65 ± 0.00^{b}	14.24 ± 0.30^{a}	18.92 ± 0.05^{a}	13.37 ± 0.40^{b}	54.90 ± 0.03^{a}	35.03 ± 1.75^{b}	97.85 ± 0.60^{a}	82.24 ± 2.96^{b}	126.17 ± 0.62^{a}	111.37 ± 0.53^{b}	
	G.A	25.10 ± 0.10	1.92 ± 0.05^{a}	1.99 ± 0.04^{a}	2.32 ± 0.02^{a}	1.97 ± 0.00^{b}	5.27 ± 0.26^{a}	2.46 ± 0.09^{b}	2.34 ± 0.06^{a}	n.d	2.88 ± 0.00^{a}	n.d	n.d	n.d	
	F.A	60.22 ± 0.40	3.38 ± 0.17^{a}	1.98 ± 0.06^{b}	6.34 ± 0.08^{a}	4.46 ± 0.01^{b}	8.13 ± 0.40^{b}	8.85 ± 0.12^{a}	13.81 ± 0.23^{a}	12.36 ± 0.47^{b}	15.49 ± 0.42^{b}	18.66 ± 0.43^{a}	17.55 ± 0.25^{b}	20.94 ± 0.79^{a}	
	C.A	25.08 ± 0.15	1.71 ± 0.01^{b}	1.85 ± 0.01^{a}	4.21 ± 0.02^{a}	2.92 ± 0.02^{b}	6.29 ± 0.10^{a}	3.39 ± 0.07^{b}	9.75 ± 0.17^{a}	4.08 ± 0.02^{b}	10.64 ± 0.31^{a}	5.73 ± 0.20^{b}	14.83 ± 0.10^{a}	14.33 ± 0.37^{a}	
	Res	7.14 ± 0.00	1.46 ± 0.01^{a}	1.47 ± 0.00^{a}	1.46 ± 0.01^{a}	1.60 ± 0.10^{a}	1.46 ± 0.00^{b}	2.67 ± 0.10^{a}	7.51 ± 0.03^{a}	4.32 ± 0.60^{b}	17.9 ± 0.61^{a}	3.34 ± 0.10^{b}	20.29 ± 0.76^{a}	13.29 ± 0.20^{b}	
70°C	Cat	53.00 ± 0.34	14.16 ± 0.56^{a}	9.53 ± 0.15^{b}	22.10 ± 0.28^{a}	20.87 ± 0.69^{a}	31.04 ± 0.14^{a}	19.42 ± 0.53b	35.34 ± 0.00^{a}	27.98 ± 0.67^{b}	32.97 ± 1.17^{a}	34.12 ± 1.47^{a}	38.09 ± 1.85^{b}	45.48 ± 0.88^{a}	
	Epi	90.22 ± 0.76	3.48 ± 0.07^{a}	2.23 ± 0.01^{b}	43.80 ± 3.14^{a}	20.56 ± 0.68^{b}	56.86 ± 0.59^{a}	22.07 ± 0.76^{b}	88.45 ± 2.34^{a}	39.41 ± 0.38^{b}	113.40 ± 1.6^{a}	61.14 ± 1.44^{b}	148.52 ± 1.45^{a}	101.98 ± 1.78^{b}	
	Epig	22.13 ± 0.89	2.07 ± 0.03^{b}	2.25 ± 0.02^{a}	15.60 ± 1.05^{a}	9.92 ± 0.28^{b}	28.96 ± 2.61^{a}	12.56 ± 0.27^{b}	22.23 ± 6.52^{a}	14.60 ± 0.51^{b}	52.24 ± 2.46^{a}	19.52 ± 0.29^{b}	43.60 ± 2.68^{a}	24.27 ± 0.94^{b}	
	EpiG	72.32 ± 0.29	n.d	42.71 ± 2.11^{a}	120.80 ± 0.18^{b}	150.79 ± 0.50^{a}	135.28 ± 0.59^{b}	184.22 ± 1.45^{a}	632.24 ± 1.26^{a}	596.29 ± 1.37^{b}	778.22 ± 15.23^{a}	488.66 ± 1.90^{b}	887.25 ± 2.45^{a}	465.78 ± 2.80^{b}	
	Pro B1	110.05 ± 0.28	3.32 ± 0.09^{b}	47.79 ± 0.09^{a}	87.20 ± 0.13^{b}	163.55 ± 2.70^{a}	116.03 ± 0.97^{b}	162.54 ± 0.90^{a}	174.62 ± 0.75^{b}	215.11 ± 0.23^{a}	228.40± 4.91^{b}	265.03 ± 0.05^{a}	173.61 ± 2.6^{b}	323.23 ± 1.60^{a}	
	Pro B2	115.32 ± 0.32	13.55 ± 0.23^{a}	7.42 ± 0.15^{b}	45.32 ± 1.04^{a}	28.90 ± 0.47^{b}	61.95 ± 0.35^{a}	48.11 ± 1.99^{b}	141.31 ± 0.13^{a}	80.02 ± 1.33^{b}	156.15 ± 0.88^{a}	124.04 ± 1.34^{b}	262.62 ± 7.04^{a}	162.79 ± 0.29^{b}	
	G.A	25.10 ± 0.10	1.93 ± 0.06^{a}	2.01 ± 0.05^{a}	2.02 ± 0.30^{a}	2.26 ± 0.08^{a}	3.06 ± 0.90^{b}	4.95 ± 0.04^{a}	7.81 ± 1.73^{a}	n.d	n.d	n.d	n.d	n.d	
	F.A	60.22 ± 0.40	1.92 ± 0.09^{b}	2.09 ± 0.05^{a}	7.60 ± 0.35^{b}	15.95 ± 0.71^{a}	9.81 ± 0.03^{b}	15.03 ± 0.61^{a}	19.59 ± 0.12^{a}	15.85 ± 0.46^{b}	24.02 ± 0.04^{a}	16.33 ± 0.04^{b}	48.40 ± 2.34^{a}	20.30 ± 0.48^{b}	
	C.A	25.08 ± 0.15	1.89 ± 0.08^{a}	1.94 ± 0.06^{a}	4.25 ± 0.25^{a}	3.33 ± 0.04^{b}	5.66 ± 0.17^{a}	3.42 ± 0.14^{b}	10.23 ± 0.29^{a}	8.72 ± 0.15^{b}	16.64 ± 0.62^{a}	12.87 ± 0.08^{b}	19.40 ± 1.04^{a}	18.27 ± 0.83^{b}	
	Res	7.14 ± 0.00	1.46 ± 0.00^{b}	1.48 ± 0.04^{a}	2.30 ± 0.02^{b}	4.74 ± 0.22^{a}	3.84 ± 0.17^{b}	5.28 ± 0.10^{a}	24.97 ± 1.48^{a}	6.64 ± 0.23^{b}	37.99 ± 0.72^{a}	9.57 ± 0.38^{b}	50.90 ± 0.68^{a}	15.26 ± 0.69^{b}	
80°C	Cat	53.00 ± 0.34	14.22 ± 0.05^{a}	9.09 ± 0.27^{b}	28.60 ± 0.17^{a}	18.63 ± 0.26^{b}	42.60 ± 0.06^{a}	27.62 ± 0.48^{b}	45.60 ± 0.00^{a}	33.73 ± 1.38^{b}	43.28 ± 0.00^{a}	41.36 ± 0.58^{b}	39.60 ± 0.00^{b}	42.70 ± 0.66^{a}	
	Epi	90.22 ± 0.76	3.48 ± 0.02^{a}	2.47 ± 0.10^{b}	48.30 ± 0.11^{a}	16.10 ± 0.76^{b}	64.80 ± 0.58^{a}	35.34 ± 0.90^{b}	94.60 ± 0.35^{a}	58.894 ± 0.79^{b}	128.30 ± 0.00^{a}	96.73 ± 0.72^{b}	158.30 ± 2.08^{a}	121.21 ± 1.92^{b}	
	Epig	22.13 ± 0.89	2.02 ± 1.50^{a}	2.39 ± 0.05^{a}	15.80 ± 2.89^{a}	9.41 ± 0.25^{b}	27.60 ± 4.62^{a}	12.56 ± 0.51^{b}	38.60 ± 4.62^{a}	17.72 ± 0.89^{b}	54.60 ± 4.62^{a}	23.32 ± 0.03^{b}	42.30 ± 4.04^{a}	26.96 ± 0.31^{b}	
	EpiG	72.32 ± 0.29	32.60 ± 0.13^{b}	39.46 ± 1.34^{a}	125.80 ± 0.81^{a}	115.29 ± 0.17^{b}	328.30 ± 1.36^{a}	206.10 ± 0.78^{b}	658.92 ± 0.35^{a}	325.30 ± 0.70^{b}	885.20 ± 2.89^{a}	672.22 ± 0.16^{b}	968.30 ± 0.81^{a}	916.22 ± 1.77^{b}	
	Pro B1	110.05 ± 0.28	3.50 ± 0.26^{b}	4.67 ± 0.11^{a}	87.20 ± 0.17^{b}	152.38 ± 1.24^{a}	116.03 ± 2.37^{b}	214.94 ± 0.46^{a}	174.62 ± 3.33^{b}	251.914 ± 1.62^{a}	228.40 ± 1.85^{b}	240.79 ± 0.81^{a}	173.61 ± 1.15^{b}	223.48 ± 1.42^{a}	
	Pro B2	115.32 ± 0.32	13.48 ± 0.03^{a}	7.28 ± 0.33^{b}	45.32 ± 0.11^{a}	32.66 ± 1.26^{b}	84.20 ± 0.70^{a}	76.38 ± 0.72^{b}	141.31 ± 0.00^{a}	125.59 ± 1.32^{b}	185.30 ± 2.89^{a}	134.75 ± 2.15^{b}	232.00 ± 0.46^{a}	111.82 ± 3.27^{b}	
	G.A	25.10 ± 0.10	1.95 ± 0.26^{a}	2.01 ± 0.04^{a}	13.30 ± 1.83^{a}	11.24 ± 0.01^{b}	4.80 ± 0.11^{a}	3.79 ± 0.15^{b}	n.d	n.d	n.d	n.d	n.d	n.d	
	F.A	60.22 ± 0.40	1.90 ± 0.07^{a}	2.08 ± 0.03^{a}	10.80 ± 0.43^{b}	12.70 ± 0.31^{a}	13.80 ± 1.36^{b}	16.99 ± 0.52^{b}	18.90 ± 0.81^{a}	17.71 ± 0.18^{a}	24.80 ± 1.73^{a}	20.02 ± 0.81^{b}	32.80 ± 1.33^{a}	23.35 ± 0.19^{b}	
	C.A	25.08 ± 0.15	1.85 ± 0.06^{a}	1.88 ± 0.01^{a}	5.20 ± 0.46^{a}	3.06 ± 0.028^{b}	12.30 ± 0.17^{a}	9.47 ± 0.22^{b}	15.70 ± 0.52^{a}	12.10 ± 0.10^{b}	22.80 ± 0.52^{a}	19.78 ± 0.78^{b}	24.80 ± 1.62^{a}	20.42 ± 0.44^{b}	
	Res	7.14 ± 0.00	1.46 ± 0.01^{a}	1.48 ± 0.01^{a}	4.50 ± 0.06^{a}	4.37 ± 0.03^{a}	6.80 ± 0.06^{b}	8.44 ± 0.39^{a}	18.90 ± 0.55^{a}	13.48 ± 0.49^{b}	35.60 ± 0.80^{a}	16.39 ± 0.41^{b}	42.30 ± 1.33^{a}	21.47 ± 0.90^{b}	

Mean (n =3) ± SD. For each maceration time from the two distinct regions, different letters in the same row indicate significant difference at $p < 0.05$Cat, catechin; Epi, epicatechin; Epig, epicatechin gallte; EpiG, epigallocatechin; Pro B1, procyanidin B1; Pro B2, procyanidin B2; G.A., gallic acid; F.A., ferulic acid; C.A., caffeic acid; Res, resveratrol; Sy-ST, Syrah Saint Thomas; Sy-F, Syrah Florentine; n.d., not detected values.

Table II.1.4-b: Flavan-3-ols and non-flavonoids profile (mg/l) of Cabernet Sauvignon musts and Cabernet Sauvignon Saint Thomas control in terms of time and temperature

		CS maceration time (hours)													
			0		2		4		8		24		48		
		Control 25°C	F	ST	F	ST	F	ST	F	ST	F	ST	F	ST	
10°C	Cat	46.02 ± 0.10	5.43 ± 0.16^{b}	7.98 ± 0.05^{a}	6.59 ± 0.10^{b}	8.82 ± 0.04^{a}	7.57 ± 0.32^{b}	9.36 ± 0.02^{a}	5.95 ± 0.03^{b}	10.43 ± 0.03^{a}	7.45 ± 0.30^{b}	11.49 ± 0.01^{a}	7.28 ± 0.16^{b}	33.51 ± 0.04^{a}	
	Epi	78.25 ± 1.01	3.13 ± 0.01^{b}	3.28 ± 0.03^{a}	5.55 ± 0.04^{a}	4.31 ± 0.01^{b}	6.83 ± 0.26^{a}	4.80 ± 0.02^{b}	5.24 ± 0.04^{a}	5.32 ± 0.02^{a}	5.66 ± 0.22^{b}	6.23 ± 0.02^{a}	6.59 ± 0.05^{b}	7.75 ± 0.01^{a}	
	Epig	29.50 ± 0.36	5.71 ± 0.12^{a}	1.88 ± 0.01^{b}	5.52 ± 0.04^{a}	1.87 ± 0.01^{b}	4.18 ± 0.18^{a}	3.20 ± 0.05^{b}	2.75 ± 0.02^{b}	3.88 ± 0.03^{a}	3.37 ± 0.02^{b}	4.54 ± 0.01^{a}	6.28 ± 0.02^{a}	5.42 ± 0.02^{b}	
	EpiG	140.20 ± 0.04	15.64 ± 2.55^{b}	27.61 ± 0.04^{a}	18.51 ± 0.56^{b}	31.83 ± 0.04^{a}	19.41 ± 0.35^{b}	40.35 ± 0.05^{a}	20.31 ± 0.21^{b}	44.28 ± 0.02^{a}	24.29 ± 0.05^{b}	65.51 ± 0.05^{a}	13.15 ± 0.55^{b}	45.8 ± 0.05^{a}	
	Pro B1	134.10 ± 1.15	11.81 ± 0.15^{a}	6.40 ± 0.02^{b}	10.36 ± 0.06^{a}	7.18 ± 0.03^{b}	15.47 ± 0.34^{a}	7.51 ± 0.01^{b}	13.58 ± 0.47^{a}	8.62 ± 0.02^{b}	6.20 ± 0.24^{b}	10.40 ± 0.03^{a}	7.78 ± 02^{b}	14.58 ± 0.02^{a}	
	Pro B2	96.45 ± 1.05	27.54 ± 1.33^{a}	11.87 ± 0.05^{b}	28.2 ± 1.06^{a}	13.29 ± 0.01^{b}	17.34 ± 0.19^{a}	13.73 ± 0.04^{b}	16.35 ± 0.28^{a}	16.25 ± 0.05^{a}	9.43 ± 0.34^{b}	23.32 ± 0.02^{a}	10.84 ± 0.11^{b}	26.93 ± 0.05^{a}	
	G.A	22.42 ± 0.17	0.65 ± 0.01^{b}	2.16 ± 0.00^{a}	0.67 ± 0.03^{b}	2.23 ± 0.05^{a}	0.67 ± 0.01^{b}	2.27 ± 0.05^{a}	0.63 ± 0.01^{b}	1.94 ± 0.03^{a}	0.66 ± 0.00^{b}	1.97 ± 0.01^{a}	1.36 ± 0.01^{b}	1.96 ± 0.01^{a}	
	F.A	20.15 ± 0.14	2.29 ± 0.05^{a}	1.63 ± 0.02^{b}	3.27 ± 0.03^{a}	1.95 ± 0.02^{b}	4.12 ± 0.10^{a}	2.43 ± 0.01^{b}	2.51 ± 0.03^{a}	2.57 ± 0.05^{a}	2.44 ± 0.02^{b}	2.85 ± 0.03^{a}	3.39 ± 0.16^{a}	3.25 ± 0.01^{a}	
	C.A	2.79 ± 0.09	2.80 ± 0.12^{a}	1.84 ± 0.01^{b}	2.08 ± 0.02^{a}	1.93 ± 0.00^{b}	2.23 ± 0.05^{a}	1.93 ± 0.02^{b}	1.77 ± 0.05^{b}	2.0 ± 0.02^{a}	2.19 ± 0.05^{a}	2.25 ± 0.02^{a}	2.34 ± 0.04^{b}	3.00 ± 0.04^{a}	
	Res	7.13 ± 0.09	1.77 ± 0.05^{a}	1.49 ± 0.05^{b}	2.03 ± 0.03^{a}	1.46 ± 0.00^{b}	0.75 ± 0.01^{b}	1.46 ± 0.00^{a}	0.74 ± 0.02^{b}	1.55 ± 0.04^{a}	0.40 ± 0.00^{b}	1.73 ± 0.01^{a}	0.39 ± 0.01^{b}	2.27 ± 0.01^{a}	
60°C	Cat	46.02 ± 0.10	6.51 ± 0.27^{b}	8.23 ± 0.01^{a}	8.50 ± 0.35^{a}	7.08 ± 0.01^{b}	10.53 ± 0.70^{b}	16.44 ± 0.02^{a}	12.81 ± 0.53^{b}	22.44 ± 0.04^{a}	22.38 ± 0.31^{b}	30.89 ± 0.01^{a}	78.14 ± 0.03^{a}	37.66 ± 0.01^{b}	
	Epi	78.25 ± 1.01	3.95 ± 0.11^{a}	3.46 ± 0.03^{b}	14.79 ± 0.31^{a}	7.15 ± 0.01^{b}	33.2 ± 0.13^{a}	11.2 ± 0.01^{b}	42.81 ± 1.45^{a}	13.45 ± 0.02^{b}	64.14 ± 2.18^{a}	33.67 ± 0.01^{b}	84.07 ± 2.00^{a}	65.91 ± 0.02^{b}	
	Epig	29.50 ± 0.36	8 .93 ± 0.02^{a}	1.86 ± 0.01^{b}	9.83 ± 0.03^{a}	4.69 ± 0.05^{b}	19.15 ± 0.04^{a}	7.62 ± 0.02^{b}	18.04 ± 0.18^{a}	9.74 ± 0.01^{b}	18.42 ± 0.23^{a}	18.22 ± 0.02^{a}	35.62 ± 0.11^{b}	48.33 ± 0.01^{a}	
	EpiG	140.20 ± 0.04	15.62 ± 1.95^{b}	27.59 ± 0.04^{a}	42.03 ± 0.34^{b}	51.6 ± 0.01^{a}	84.12 ± 1.99^{a}	62.24 ± 0.01^{b}	118.85 ± 2.77^{a}	94.75 ± 0.03^{b}	137.48 ± 1.01^{a}	107.57 ± 0.03^{b}	193.22 ± 1.02^{a}	105.40 ± 0.03^{b}	
	Pro B1	134.10 ± 1.15	4.49 ± 0.07^{b}	6.26 ± 0.01^{a}	10.90 ± 0.06^{a}	9.16 ± 0.01^{b}	18.37 ± 0.05^{a}	12.42 ± 0.05^{b}	24.43 ± 0.11^{a}	21.59 ± 0.05^{b}	104.86 ± 0.16^{b}	112.76 ± 0.03^{a}	72.72 ± 0.25^{a}	55.21 ± 0.01^{b}	
	Pro B2	96.45 ± 1.05	24.92 ± 0.11^{a}	12.09 ± 0.04^{b}	32.25 ± 0.06^{a}	17.29 ± 0.05^{b}	39.27 ± 0.44^{a}	22.13 ± 0.05^{b}	29.84 ± 0.13^{b}	36.37 ± 0.02^{a}	34.10 ± 1.43^{b}	66.84 ± 0.01^{a}	287.34 ± 5.83^{a}	138.80 ± 0.03^{b}	
	G.A	22.42 ± 0.17	0.65 ± 0.01^{b}	2.08 ± 0.05^{a}	0.67 ± 0.01^{b}	2.07 ± 0.01^{a}	1.14 ± 0.00^{b}	2.34 ± 0.03^{a}	2.12 ± 0.00^{b}	3.26 ± 0.01^{a}	2.46 ± 0.01^{b}	3.56 ± 0.01^{a}	n.d	n.d	
	F.A	20.15 ± 0.14	3.1 ± 0.04^{a}	2.27 ± 0.03^{b}	6.10 ± 0.17^{a}	3.02 ± 0.05^{b}	3.49 ± 0.00^{a}	2.72 ± 0.01^{b}	6.16 ± 0.25^{b}	7.49 ± 0.01^{a}	10.46 ± 0.31^{b}	14.54 ± 0.01^{a}	18.81 ± 0.06^{a}	13.70 ± 0.04^{b}	
	C.A	2.79 ± 0.09	2.93 ± 0.01^{a}	1.84 ± 0.01^{b}	4.04 ± 0.02^{a}	2.07 ± 0.05^{b}	2.83 ± 0.03^{a}	2.42 ± 0.03^{b}	2.63 ± 0.05^{b}	5.12 ± 0.01^{a}	4.23 ± 0.12^{b}	9.19 ± 0.01^{a}	6.42 ± 0.28^{b}	13.60 ± 0.04^{a}	
	Res	7.13 ± 0.09	1.69 ± 0.01^{a}	1.46 ± 0.00^{b}	6.19 ± 0.10^{a}	1.55 ± 0.02^{b}	6.22 ± 0.02^{a}	2.12 ± 0.05^{b}	13.24 ± 0.01^{a}	3.17 ± 0.05^{b}	23.3 ± 0.05^{a}	7.77 ± 0.02^{b}	53.33 ± 0.05^{a}	29.64 ± 0.03^{b}	
70°C	Cat	46.02 ± 0.10	6.57 ± 0.01^{b}	8.18 ± 0.01^{a}	13.35 ± 0.20^{a}	13.63 ± 0.04^{a}	15.44 ± 1.42^{b}	18.24 ± 0.01^{a}	20.44 ± 0.40^{b}	67.20 ± 0.02^{a}	24.90 ± 0.50^{b}	85.56 ± 0.02^{a}	62.05 ± 2.90^{b}	92.64 ± 0.01^{a}	
	Epi	78.25 ± 1.01	4.84 ± 0.03^{a}	3.48 ± 0.05^{b}	52.00 ± 1.01^{a}	15.64 ± 0.01^{b}	79.50 ± 2.52^{a}	21.41 ± 0.04^{b}	83.27 ± 1.86^{a}	74.03 ± 0.02^{b}	121.55 ± 1.02^{a}	82.59 ± 0.02^{b}	132.15 ± 2.53^{a}	101.61 ± 0.02^{b}	
	Epig	29.50 ± 0.36	8.39 ± 0.26^{a}	1.87 ± 0.00^{b}	9.32 ± 0.13^{b}	12.29 ± 0.03^{a}	19.53 ± 0.13^{a}	13.74 ± 0.04^{b}	23.70 ± 0.31^{a}	18.18 ± 0.06^{b}	32.78 ± 1.53^{a}	32.13 ± 0.02^{a}	42.61 ± 1.80^{b}	64.61 ± 0.01^{a}	
	EpiG	140.20 ± 0.04	15.68 ± 0.78^{b}	27.82 ± 0.08^{a}	165.23 ± 2.56^{a}	97.36 ± 0.02^{b}	164.09 ± 1.38^{a}	141.41 ± 0.01^{b}	200.80 ± 0.05^{b}	456.26 ± 0.04^{a}	220.48 ± 2.41^{b}	556.97 ± 0.05^{a}	232.61 ± 2.84^{b}	566.74 ± 0.04^{a}	
	Pro B1	134.10 ± 1.15	8.36 ± 0.17^{a}	6.41 ± 0.01^{a}	20.01 ± 1.80^{b}	25.43 ± 0.05^{a}	45.35 ± 1.45^{b}	50.54 ± 0.01^{a}	25.24 ± 0.65^{b}	173.11 ± 0.03^{a}	75.04 ± 2.89^{b}	279.59 ± 0.01^{a}	115.03 ± 3.68^{b}	179.17 ± 0.06^{a}	
	Pro B2	96.45 ± 1.05	24.51 ± 0.22^{a}	12.31 ± 0.02^{b}	74.35 ± 1.07^{a}	24.59 ± 0.02^{b}	66.04 ± 0.03^{a}	34.18 ± 0.05^{b}	75.01 ± 1.16^{b}	99.14 ± 0.05^{a}	91.34 ± 0.034^{b}	144.21 ± 0.04^{a}	372.14 ± 0.01^{a}	133.85 ± 0.02^{b}	
	G.A	22.42 ± 0.17	0.65 ± 0.01^{b}	2.14 ± 0.04^{a}	2.43 ± 0.03^{b}	3.64 ± 0.05^{a}	2.36 ± 0.00^{b}	2.04 ± 0.00^{a}	4.48 ± 0.01^{b}	9.67 ± 0.01^{a}	2.50 ± 0.05^{b}	8.14 ± 0.01^{a}	n.d	n.d	
	F.A	20.15 ± 0.14	2.95 ± 0.03^{a}	2.25 ± 0.00^{b}	13.41 ± 0.21^{a}	7.58 ± 0.02^{b}	17.48 ± 0.43^{a}	7.32 ± 0.02^{b}	18.79 ± 0.13^{a}	12.62 ± 0.05^{b}	11.79 ± 0.13^{b}	15.19 ± 0.01^{a}	12.37 ± 0.36^{b}	22.17 ± 0.01^{a}	
	C.A	2.79 ± 0.09	2.91 ± 0.04^{a}	1.87 ± 0.00^{b}	5.65 ± 0.16^{a}	2.34 ± 0.05^{b}	5.10 ± 0.14^{b}	5.33 ± 0.01^{a}	5.60 ± 0.05^{b}	6.38 ± 0.01^{a}	5.52 ± 0.21^{b}	10.78 ± 0.05^{a}	8.39 ± 0.25^{b}	15.64 ± 0.02^{a}	
	Res	7.13 ± 0.09	1.68 ± 0.02^{a}	1.46 ± 0.00^{b}	12.46 ± 0.06^{a}	2.10 ± 0.05^{b}	16.22 ± 0.15^{a}	2.45 ± 0.03^{b}	23.11 ± 0.07^{a}	5.86 ± 0.01^{b}	33.33 ± 0.05^{a}	15.35 ± 0.01^{b}	50.70 ± 0.25^{a}	44.38 ± 0.04^{b}	
80°C	Cat	46.02 ± 0.10	5.35 ± 0.26^{b}	8.11 ± 0.04^{a}	12.96 ± 1.43^{b}	20.25 ± 0.02^{a}	25.14 ± 2.27^{b}	37.62 ± 0.01^{a}	24.78 ± 0.94^{b}	106.62 ± 0.03^{a}	26.65 ± 0.83^{b}	97.12 ± 0.04^{a}	64.41 ± 2.35^{b}	81.22 ± 0.02^{a}	
	Epi	78.25 ± 1.01	4.94 ± 0.19^{a}	3.61 ± 0.01^{b}	86.02 ± 3.68^{a}	22.62 ± 0.03^{b}	103.08 ± 4.11^{a}	42.63 ± 0.01^{b}	112.82 ± 2.51^{b}	143.60 ± 0.01^{a}	124.07 ± 0.63^{a}	105.48 ± 0.02^{b}	104.56 ± 0.52^{a}	106.26 ± 0.03^{a}	
	Epig	29.50 ± 0.36	6.42 ± 0.14^{a}	1.88 ± 0.01^{b}	11.38 ± 0.25^{b}	14.00 ± 0.03^{a}	39.50 ± 0.39^{a}	23.85 ± 0.05^{b}	39.76 ± 1.15^{a}	23.18 ± 0.05^{b}	39.32 ± 0.76^{b}	94.52 ± 0.05^{a}	57.70 ± 2.09^{b}	156.15 ± 0.02^{a}	
	EpiG	140.20 ± 0.04	15.67 ± 0.82^{b}	27.97 ± 0.04^{a}	170.77 ± 2.85^{b}	547.62 ± 0.05^{a}	283.10 ± 3.28^{b}	858.32 ± 0.01^{a}	177.34 ± 1.59^{b}	937.31 ± 0.02^{a}	96.3 ± 2.34^{b}	984.73 ± 0.01^{a}	95.55 ± 2.20^{b}	695.25 ± 0.02^{a}	
	Pro B1	134.10 ± 1.15	11.89 ± 0.46^{a}	6.64 ± 0.01^{b}	53.73 ± 4.91^{b}	104.03 ± 0.02^{a}	72.38 ± 3.48^{b}	147.41 ± 0.05^{a}	82.24 ± 0.34^{a}	65.92 ± 0.02^{b}	96.47 ± 2.77^{b}	254.15 ± 0.05^{a}	100.04 ± 3.42^{b}	155.01 ± 0.01^{a}	
	Pro B2	96.45 ± 1.05	29.33 ± 0.54^{a}	12.58 ± 0.01^{b}	96.29 ± 4.45^{a}	44.30 ± 0.05^{b}	169.69 ± 5.87^{a}	86.12 ± 0.02^{b}	191.49 ± 2.70^{a}	47.73 ± 0.01^{b}	197.32 ± 3.37^{a}	184.58 ± 0.04^{b}	328.76 ± 1.14^{a}	183.36 ± 0.02^{b}	
	G.A	22.42 ± 0.17	0.65 ± 0.01^{b}	2.16 ± 0.01^{a}	3.07 ± 0.02^{b}	6.69 ± 0.02^{a}	3.37 ± 0.11^{b}	6.88 ± 0.04^{a}	3.43 ± 0.04^{b}	13.87 ± 0.05^{a}	8.85 ± 0.41^{a}	0.00 ± 0.00^{b}	n.d	0.00 ± 0.00^{b}	
	F.A	20.15 ± 0.14	2.67 ± 0.02^{a}	1.51 ± 0.03^{b}	18.18 ± 0.07^{a}	10.38 ± 0.02^{b}	21.30 ± 0.69^{a}	12.60 ± 0.02^{b}	21.69 ± 0.25^{a}	12.32 ± 0.03^{b}	21.45 ± 0.40^{a}	14.44 ± 0.04^{b}	20.12 ± 0.05^{a}	15.66 ± 0.01^{b}	
	C.A	2.79 ± 0.09	2.95 ±0.035^{a}	1.88 ± 0.00^{b}	5.20 ± 0.17^{a}	2.46 ± 0.04^{b}	5.62 ± 0.07^{b}	8.39 ± 0.02^{a}	5.31 ± 0.18^{b}	9.12 ± 0.01^{a}	7.50 ± 0.22^{b}	16.64 ± 0.04^{a}	11.51 ± 0.32^{b}	19.51 ± 0.04^{a}	
	Res	7.13 ± 0.09	1.88 ± 0.05^{a}	1.46 ± 0.00^{b}	13.34 ± 0.37^{a}	2.98 ± 0.01^{b}	16.19 ± 0.09^{a}	6.63 ± 0.01^{b}	32.54 ± 0.038^{a}	12.53 ± 0.04^{b}	42.24 ± 0.02^{a}	21.23 ± 0.01^{b}	45.96 ± 0.04^{a}	29.23 ± 0.03^{b}	

Mean (n =3) ± SD. For each maceration time from the two distinct regions, different letters in the same row indicate significant difference at $p < 0.05$Cat, catechin; Epi, epicatechin; Epig, epicatechin gallte; EpiG, epigallocatechin; Pro B1, procyanidin B1; Pro B2, procyanidin B2; G.A., gallic acid; F.A., ferulic acid; C.A., caffeic acid; Res, resveratrol; CS-ST, Cabernet Sauvignon Saint Thomas; CS-F, Cabernet Sauvignon Florentine; n.d., not detected values.

II.1.3.2. IMPACT OF MACERATION TIME AND TEMPERATURE ON BIOLOGICAL ACTIVITIES

By comparing the different biological activities found in the different grape must after 48 h of maceration at different temperatures (10°C, 60°C, 70°C, 80°C and 25°C), Figure II.1.3-a and II.1.3-b demonstrated that temperatures of 10°C and 60°C showed remarkably low biological activities for the different musts compared to 70°C and 80°C from Syrah Saint Thomas. The same low activities were noticed for CS-F macerated at 80°C after 48 hours. Sy-ST macerated at 70°C exhibited the highest inhibition percentage for the most of the biological activities studied. The ABTS, DPPH, LOX, α- glucosidase and HCT116 values were respectively 63.31; 52.48; 84.6; 35.7and 47.6%. These values are 3 times higher for ABTS and DPPH and 18 times higher for LOX than for Sy-F at the same temperature.

This can be due as seen in Figure II.1.5-a to the highest content of Sy-ST-70°C on procyanidin B1 and catechin, or other phenolic compounds which have not been analyzed. Furthermore, Sy-ST control had 1.46 times higher antidiabetic activities than Sy-ST macerated at 70°C which may be the result of it is high anthocyanin and gallic acid content compared to Syrah musts (Figure II.1.5-a). These compounds according to the other studies (Sri Balasubashini et al., 2003 and Zunino, 2009) have been shown to inhibit hyperglycemia. Among Cabernet Sauvignon must, CS-ST-70°C, showed the highest inhibition‟s percentage for ABTS, DPPH, LOX, and α-glucosidase (24.19; 19.15; 27.21; 9.49; 5.98% respectively), but these values were 2.65 times lower for ABTS and DPPH; 3.10 times lower for LOX; 3.76 times lower for α-glucosidase; 1.11 times lower for ChE and 14.42 times lower for HCT116 than for Sy-ST. So as seen in Figure II.3-a, Sy-ST-70°C exhibited the highest activities among must samples.

CS-ST control showed 2.41 and 5.51 times higher anti-LOX and anti α-glucosidase respectively than CS-ST-70°C which can be due as mentioned above to it is high content in gallic acid. In fact phenolic acids provide meaningful synergistic protection against hypoglycemic and anti-inflammatory effects (Sri Balasubashini et al., 2003; Yagi and Ohishi, 1979). So, this could be explained by the fact that not all phenolics compounds had the same contribution to the antioxidant activity. Many reports have shown that the antioxidant potentiel of final foodstuff depends on the qualitative and quantitative composition of polyphenols in raw material (Rice-Evans et al., 1997; Owczarek et al., 2004). In addition, Study conducted by Lingua et al. (2016) demonstrated that in case of grapes, astilbin and procyanidin dimer were compounds with highest positive contribution to the FRAP, ABTS and DPPH value, while peonidin-3-

coumaroylglucoside, (-)-epicatechin and myricetin were the ones with highest negative contribution. Furthermore, other natural antioxidant present in the grapes especially viniferin, quercetin, and catechin play an important role in inflammatory disorders (Leifert and Abeywardena, 2008). Besides, Resveratrol suppresses proliferation of a wide variety of tumor cells, including lymphoid, myeloid, breast, prostate, stomach, colon, pancreas, thyroid, skin, head and neck, ovarian, and cervical (Jacquelyn and John, 2011).

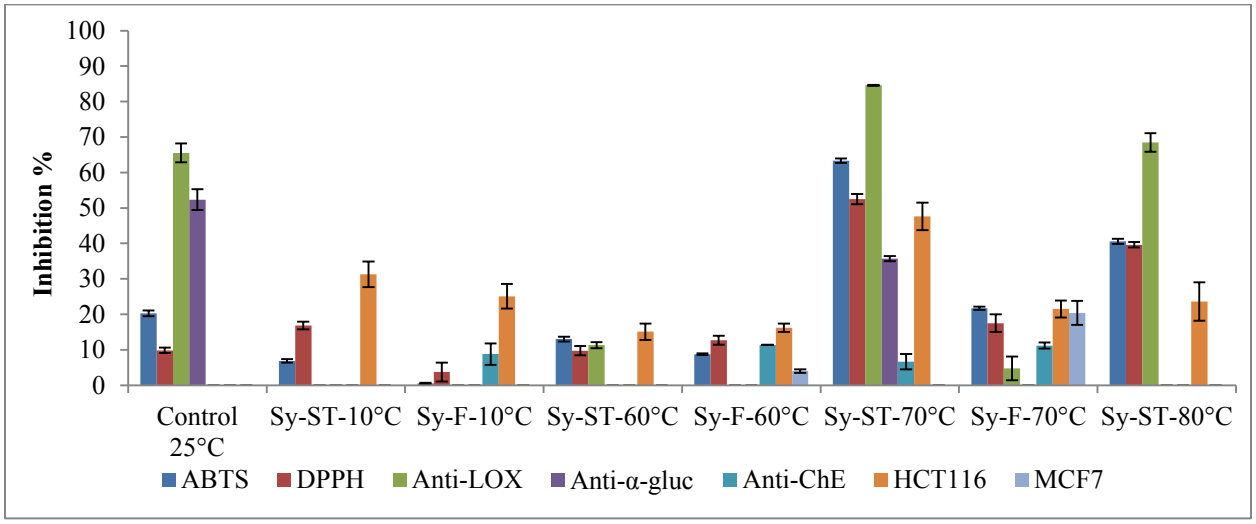

Figure II.1.3-a: Biological activities (ABTS and DPPH (antioxidant), Anti-LOX (antiinflammatory), Anti-α gluc (antidiabetic), Anti-ChE (antialzheimer), HCT116 and MCF7 (anticancer)) of Sy-ST (Syrah Saint Thomas) and Sy-F (Syrah Florentine) grape musts macerated at different temperatures (10°C, 60°C, 70°C, 80°C) after 48 hours and for the control (Sy-ST-25°C) after alcoholic fermentation. Data were expressed as mean (n=3) percentage of inhibition (inhibition %) ± standard deviation

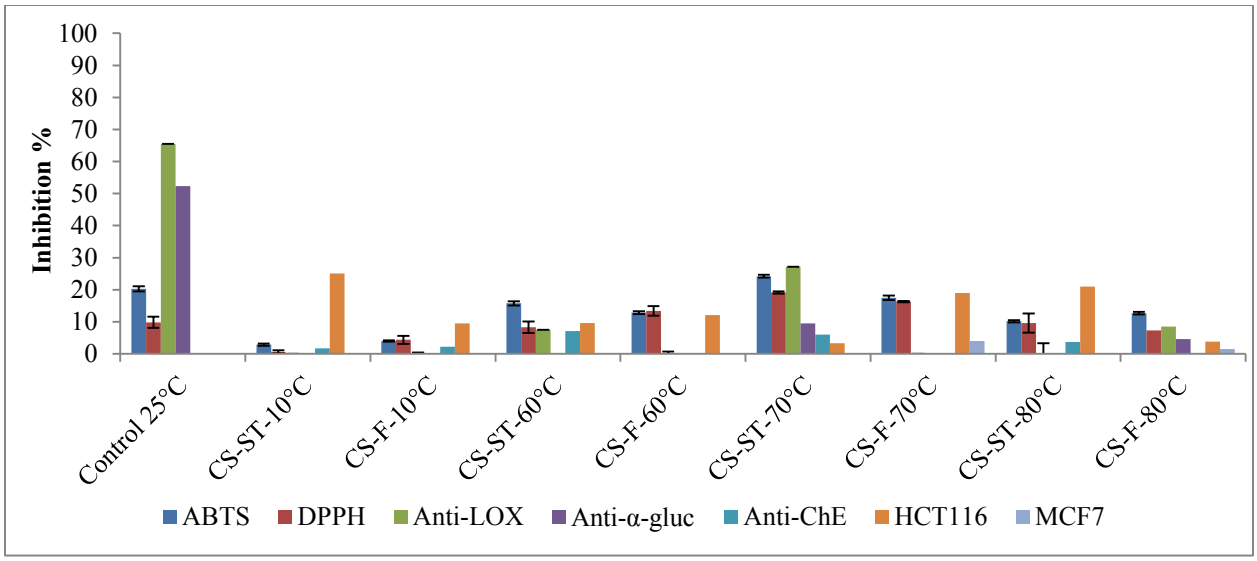

Figure II.1.3-b: Biological activities (ABTS and DPPH (antioxidant), Anti-LOX (antiinflammatory), Anti-α gluc (antidiabetic), Anti-ChE (antialzheimer), HCT116 and MCF7 (anticancer)) of CS-ST (Cabernet Sauvignon Saint Thomas) and CS-F (Cabernet Sauvignon Florentine) musts macerated at different temperatures (10°C, 60°C, 70°C, 80°C) after 48 hours and for the control (CS-ST-25°C) after alcoholic fermentation. Data were expressed as mean (n=3) percentage inhibition (inhibition %) ± standard deviation

II.1.4. Effect of Terroir

Since the effect of grape varieties within the same terroir is already known we do not go deeper into details. Figure II.1.4 showed the PCA biplot for the first two principal component analyses obtained from the colour and phenolic composition of Syrah and Cabernet Sauvignon Saint Thomas musts which explain 79.37% of the total variance. The first component is positively represented by the variables TA, CI, TPI, TP, T, ABTS, Dp, Pro B1, EpiG, Cat, ProB2, C.A, Epi, Epig, F.A and Res. The second component is positively represented by Cy, Pn and Mv. The projection of the Syrah and Cabernet Sauvignon Saint Thomas must samples over maceration time (0, 2, 4, 8, 24 and 48 h) at different temperatures (10°C, 60°C, 70°C, and 80°C) showed that Cabernet Sauvignon had the highest content of total polyphenols, this effect was more important with increasing maceration time and temperatures (Figure II.1.4). So within the same terroir we have the effect of grape varieties. These results are in agreement with those reported by Lingua et al. (2016).

In order to examine the impact of Terroir, Figure II.1.5-a and II.1.5-b represented respectively the evolution of Syrah and Cabernet Sauvignon musts from the two different regions over maceration time compared to Syrah control at the end of alcoholic fermentation. Figure II.1.5-a showed the PCA biplot for the first two principal component analysis obtained from the color and phenolic composition of Sy-F and Sy-ST musts which explain 82.17% of the total variance. The first component is positively represented by the variables TA, CI, TPI, TP, T, ABTS, Pro B1, EpiG, Cat, ProB2, CA, Epi, Epig, FA and Res. The second component is positively represented by Dp, Cy, Pn, Mv and GA, While Figure II.1.5-b showed the PCA biplot for the first two principal component analysis obtained from the color and phenolic composition of CS-F and CS-ST musts which which explain 72.73% of the total variance. The first component was positively represented by the variables TA, CI, TPI, TP, T, ABTS, Dp, Cy, GA, Pro B1, EpiG, Cat, ProB2, CA, Epi, Epig, FA and Res. The second component was positively represented by Pn and Mv.

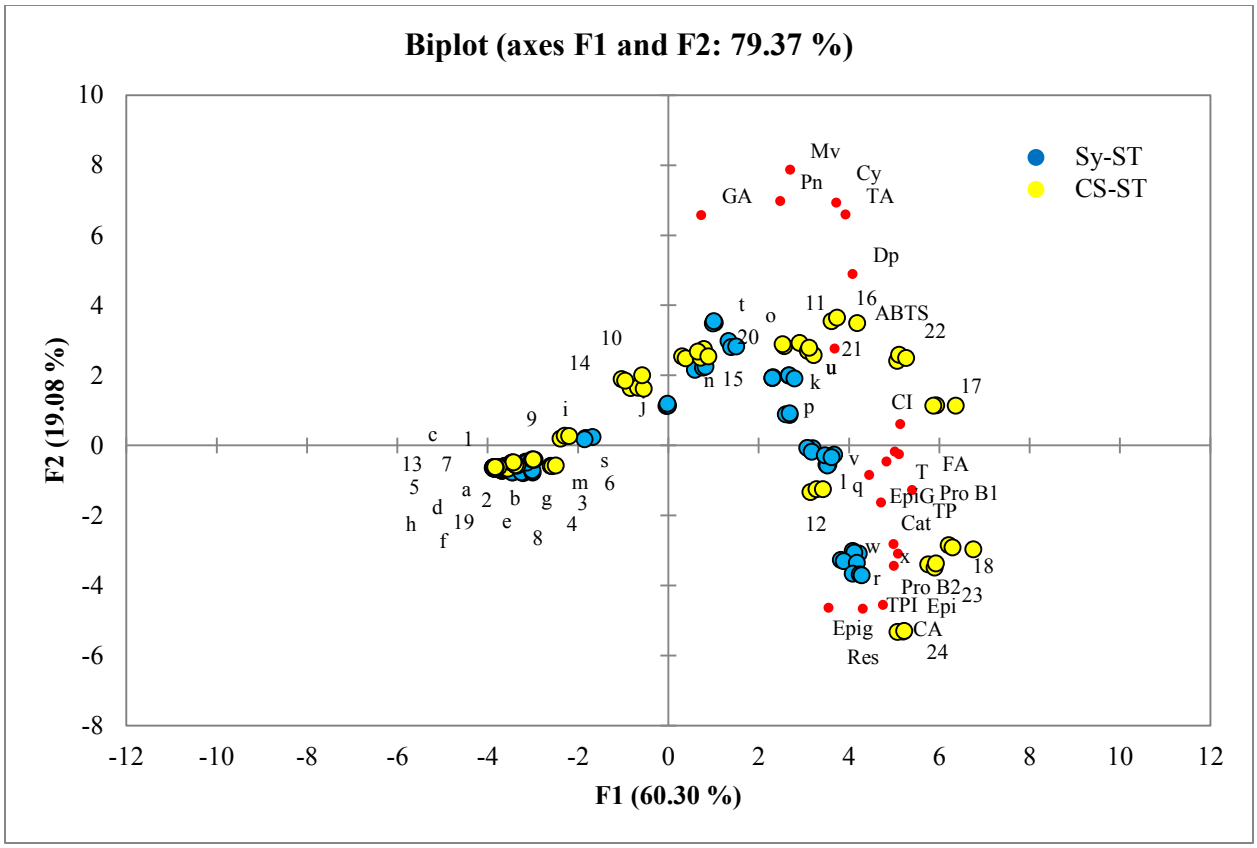

Figure II.1.4: Biplot of the two first principal components obtained from the colour and phenolic composition of Sy-ST (Syrah Saint Thomas) and CS-ST (Cabernet Sauvignon Saint Thomas) musts: TA, total anthocyanin content; CI, color intensity; TPI, total polyphenol index; TP, total polyphenols; T, Tannins; ABTS, Dp, delphinidin-3-O-glucoside ; Cy, cyanidin-3-O-glucoside ; Pn, peonidin-3-O-glucoside ; Mv, malvidin-3-O-glucoside ; GA, gallic acid; pro B1, procyanidin B1; EpiG, epigallocatechin; cat, catechin; Pro B2, procyanidin B2; CA, caffeic acid; Epi, epicatechin; Epig, epicatechin gallate; FA, ferulic acid; Res; resveratrol; obtained after maceration at different temperatures (10, 60, 70 and 80°C) for 48 hours (a, Sy-ST-0-10°C; b, Sy-ST-2-10°C, c, Sy-ST-4-10°C; d, Sy-ST-8-10°C; e, Sy-ST-24-10°C; f, Sy-ST-48-10°C; g, Sy-ST-0-60°C; h, Sy-ST-2-60°C; i, Sy-ST-4-60°C; j, Sy-ST-8-60°C; k, Sy-ST-24-60°C; l, Sy-ST-48-60°C; m, Sy-ST-0-70°C; n, Sy-ST-2-70°C; o, Sy-ST-4-70°C; p, Sy-ST-8-70°C; q, Sy-ST-24-70°C; r, Sy-ST-48-70°C; s, Sy-ST-0-80°C; t, Sy-ST-2-80°C; u, Sy-ST-4-80°C; v, Sy-ST-8-80°C; w, Sy-ST-24-80°C; x, Sy-ST-48-80°C; 1, CS-ST-0-10°C; 2, CS-ST-2-10°C; 3, CS-ST-4-10°C; 4, CS-ST-8-10°C; 5, CS-ST-24-10°C; 6, CS-ST-48-10°C; 7, CS-ST-0-60°C; 8, CS-ST-2-60°C; 9, CS-ST-4-60°C; 10, CS-ST-8-60°C; 11, CS-ST-24-60°C; 12, CS-ST-48-60°C; 13, CS-ST-0-70°C; 14, CS-ST-2-70°C; 15, CS-ST- 4-70°C; 16, CS-ST-8-70°C; 17, CS-ST-24-70°C; 18, CS-ST-48-70°C; 19, CS-ST-0-80°C; 20, CS-ST-2-80°C; 21, CS-ST-4-80°C; 22, CS-ST-8-80°C; 23, CS-ST-24-80°C; 24, CS-ST-48-80°C).

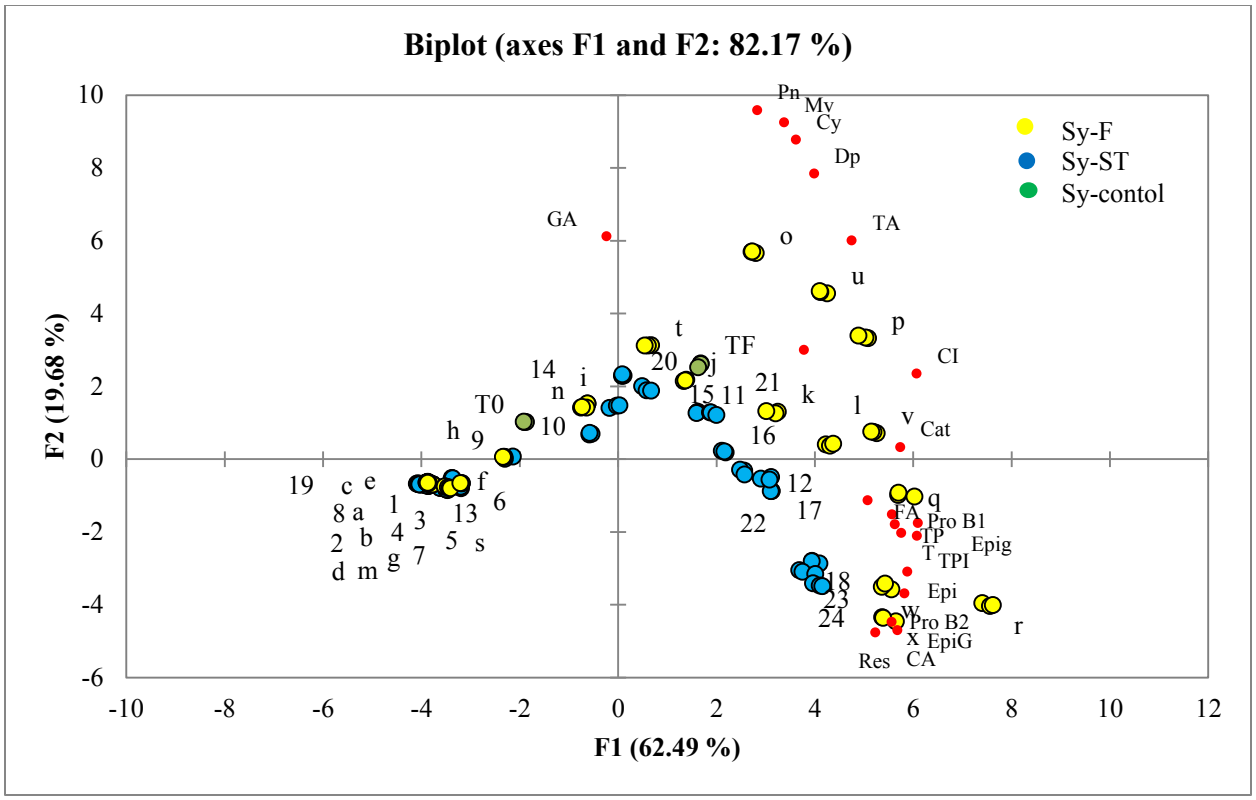

Figure II.1.5-a: Biplot of the two first principal components obtained from the colour and phenolic composition of Sy-F (Syrah Florentine) and Sy-ST (Syrah Saint Thomas) musts compared to Syrah Saint Thomas control (Sy-control): TA, total anthocyanin content; CI, color intensity; TPI, total polyphenol index; TP, total polyphenols; T, Tannins; ABTS, Dp, delphinidin-3-O-glucoside ; Cy, cyanidin-3-O-glucoside ; Pn, peonidin-3-O-glucoside ; Mv, malvidin-3-O-glucoside ; GA, gallic acid; pro B1, procyanidin B1; EpiG, epigallocatechin; cat, catechin; Pro B2, procyanidin B2; CA, caffeic acid; Epi, epicatechin; Epig, epicatechin gallate; FA, ferulic acid; Res; resveratrol; obtained after maceration at different temperatures (10, 60, 70 and 80°C) for 48 hours (a, Sy-F-0-10°C; b, Sy-F-2-10°C, c, Sy-F-4-10°C; d, Sy-F-8-10°C; e, Sy-F-24-10°C; f, Sy-F-48-10°C; g, Sy-F-0-60°C; h, Sy-F-2-60°C; i, Sy-F-4-60°C; j, Sy-F-8-60°C; k, Sy-F-24-60°C; l, Sy-F-48-60°C; m, Sy-F-0-70°C; n, Sy-F-2-70°C; o, Sy-F-4-70°C; p, Sy-F-8-70°C; q, Sy-F-24-70°C; r, Sy-F-48-70°C; s, Sy-F-0-80°C; t, Sy-F-2-80°C; u, Sy-F-4-80°C; v, Sy-F-8-80°C; w, Sy-F-24-80°C; x, Sy-F-48-80°C; 1, Sy-ST-0-10°C; 2, Sy-ST-2-10°C; 3, Sy-ST-4-10°C; 4, Sy-ST-8-10°C; 5, Sy-ST-24-10°C; 6, Sy-ST-48-10°C; 7, Sy-ST-0-60°C; 8, Sy-ST-2-60°C; 9, Sy-ST-4-60°C; 10, Sy-ST-8-60°C; 11, Sy-ST-24-60°C; 12, Sy-ST-48-60°C; 13, Sy-ST-0-70°C; 14, Sy-ST-2-70°C; 15, Sy-ST-4-70°C; 16, Sy-ST-8-70°C; 17, Sy-ST-24-70°C; 18, Sy-ST-48-70°C; 19, Sy-ST-0-80°C; 20, Sy-ST-2-80°C; 21, Sy-ST-4-80°C; 22, Sy-ST-8-80°C; 23, Sy-ST-24-80°C; 24, Sy-ST-48-80°C; To, Syrah control at the beginning of maceration ; TF, Syrah control at the end of alcoholic fermentation.

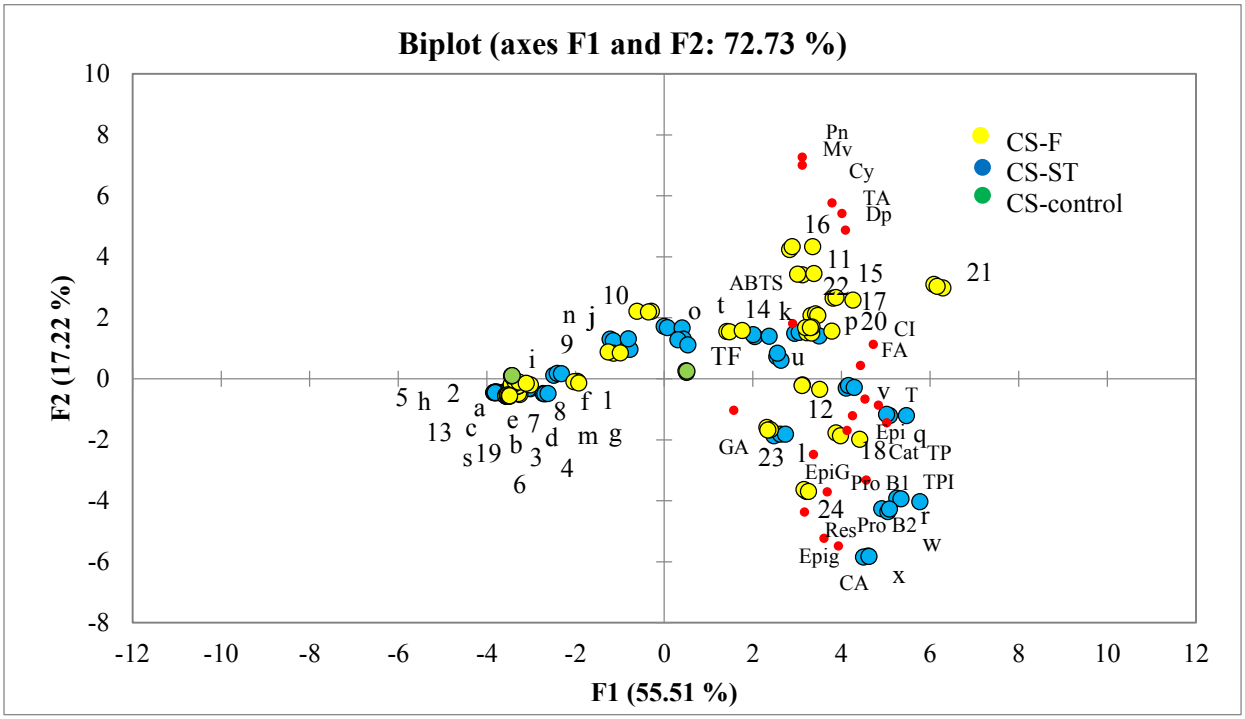

Figure II.1.5-b: Biplot of the two first principal components obtained from the colour and phenolic composition of the CS-F (Cabernet Sauvignon Florentine) and CS-ST (Cabernet Sauvignon Saint Thomas) red musts compared to Cabernet Sauvignon Saint Thomas wines control (CS-control): TA, total anthocyanin content; CI, color intensity; TPI, total polyphenol index; TP, total polyphenols; T, Tannins; ABTS, Dp, delphinidin-3-O-glucoside ; Cy, cyanidin-3-O-glucoside ; Pn, peonidin-3-O-glucoside ; Mv, malvidin-3-O-glucoside ; GA, gallic acid; pro B1, procyanidin B1; EpiG, epigallocatechin; cat, catechin; Pro B2, procyanidin B2; C.A, caffeic acid; Epi, epicatechin; Epig, epicatechin gallate; F.A, ferulic acid; Res; resveratrol; obtained after maceration at different temperatures for 48 hours (1, CS-F-0-10°C; 2, CS-F-2-10°C; 3, CS-F-4-10°C; 4, CS-F-8-10°C; 5, CS-F-24-10°C; 6, CS-F-48-10°C; 7, CS-F-0-60°C ; 8, CS-F-2-60°C ; 9, CS-F-4-60°C ; 10, CS-F-8-60°C ; 11, CS-F-24-60°C ; 12, CS-F-48-60°C ; 13, CS-F-0-70°C ; 14, CS-F-2-70°C ; 15, CS-F-4-70°C ; 16, CS-F-8-70°C ; 17, CS-F-24-70°C ; 18, CS-F-48-70°C ; 19, CS-F-0-80°C ; 20, CS-F-2-80°C ; 21, CS-F-4-80°C ; 22, CS-F-8-80°C ; 23, CS-F-24-80°C ; 24, CS-F-48-80°C ; a, CS-ST-0-10°C; b, CS-ST-2-10°C; c, CS-ST-4-10°C; d, CS-ST-8-10°C; e, CS-ST-24-10°C; f, CS-ST-48-10°C; g, CS-ST-0-60°C ; h, CS-ST-2-60°C ; i, CS-ST-4-60°C ; j, CS-ST-8-60°C ; k, CS-ST-24-60°C ; l, CS-ST-48-60°C ; m, CS-ST-0-70°C ; n, CS-ST-2-70°C ; o, CS-ST-4-70°C ; p, CS-ST-8-70°C ; q, CS-ST-24-70°C ; r, CS-ST-48-70°C ; s, CS-ST-0-80°C ; t, CS-ST-2-80°C ; u, CS-ST-4-80°C ; v, CS-ST-8-80°C ; w, CS-ST-24-80°C ; x, CS-st-48-80°C; To, control at the beginning of maceration ; TF, control at the end of alcoholic fermentation.

The projection of the Syrah Saint Thomas and Syrah Florentine must samples (Figue II.1.5-a) over maceration time (0, 2, 4, 8, 24 and 48 h) at different temperatures (10°C, 60°C, 70°C, and 80°C) showed similar evolution of the two musts over time with a higher concentration of total phenolic compounds for Syrah Florentine than for Syrah Saint Thomas, suggesting that the accumulation of phenolic compounds in grape berries is strongly affected by „‟terroir‟‟‟ factors (Gambelli and Santorini, 2004; pereira et al., 2006). The results showed (Table II.1.4-a), that grape must collected from Majdel Meouch vineyard demonstrated the significantly highest global average values of flavonoid and non-flavonoid conpounds. In addition, The projection of the CS Saint Thomas and CS Florentine must samples (Figure II.1.5-b) over maceration time (0, 2, 4, 8, 24 and 48 h) at different temperatures (10°C, 60°C, 70°C, and 80°C) showed similar evolution of the two musts over time.

Studies in the literature showed that during ripeness period grapes suffered high differences of temperatures between day and night which could justify the high anthocyanin content (Mateus et al., 2001; Yamane et al., 2006). Also, previous researches showed that light, water deficits and higher temperature differences between daytime and nighttime could up-regulate the gene expression related to flavonoid metabolism, and thus significantly increase the contents of flavonoid (Gollop et al., 2002; Yamane et al., 2006). Infertile soil, rather than fertile ones, provides with more composite and content of inorganic ions, activating flavonoid synthesis (Boulton, 1980; Reeve et al., 2005). All the cited factors are in accordance with the data of Clos Saint Thomas rather than Chateau Florentine. This observation is contradictory with the obtained results where the musts of Chateau Florentine showed higher concentrations in polyphenols than those of Clos Saint Thomas. Other factors could play an important role as training system of the vines, fertilization of soils, irrigation during summer and canopy management.

As regards to stilbenes, Resveratrols is a phenolic phytoalexin produced by grapevines in response to fungal infection and stress. Studies report a role of resveratrol especially in the prevention of cardiovascular disease. The amounts varied depending on many factors such climatic and agronomic factors. Sy-F showed the highest level of trans-resveratrol content, which can be explained both by the climatic and soil factors. Majdel Meoouch‟s climate is classified as humid climate and according to studies conducted by Kolouchova-Hanzlikova et al. 2004 Cooler and more humid climatic conditions lead to higher trans-resveratrol content.

In fact, soil effect on stilbene amount has been proved to be as important as climate effect (Andres de Prado et al., 2007). Florentine had clayey soil texture; these soils have a very high water-holding capacity which favors rot development leading to higher trans-resveratrol content. These results are in accordance with previous published results of (Andres de Prado et al., 2007; Koundouras et al., 2006; Bavaresco et al., 2009), which described that soils with high water-holding capacity might stimulate stilbene biosynthesis in grape.

Moreover, Syrah can be suggested as one of the most suitable varieties for obtaining stilbene-enriched wines, in agreement with previous results (Guerrero et al., 2010) and Florentine type terroir as accurate terroir for it is cultivation, in order to obtain enriched wines with stilbenes with added value. On the contrary the terroir effect for Cabernet Sauvignon musts was less important than those of Syrah musts this can be explained by the fact that for this variety higher maceration temperatures masked terroir effects. Eventually, while tannins was progressively extracted from skins and seeds, the potential of anthocyanins was extracted since the first hours, so temperature and length of maceration are parameters that must be adjusted to grape varieties and defined terroirs. Figure II.1.5 allowed establishing the best couple time/temperature for each grape must without degradation kinetics of anthocyanins and gallic acid over time. This couple was represented by the letter v (Figure II.1.5-a) corresponding to Sy-F-8-80°C, the number 16 and 12 (Figure II.1.5-a) corresponding respectively to Sy-ST-8-70°C and Sy-ST- 48-60°C, the number 12 corresponding to CS-F-48-60°C (Figure II.1.5-b) and the letter v corresponding to CS-ST-8-80°C (Figure II.1.5-b)

II.1.5. Conclusion

The results presented in this study highlight that the phenolic composition of musts is greatly affected by the maceration step. The pre-fermentation heat treatment of grapes is more efficient for the extraction of polyphenols than the cold maceration. Analysis of must samples revealed a systematic increase in the concentration of tannins with temperature and over time.

Temperature favored anthocyanin extraction, a degradation of these compounds was observed at high temperatures when the maceration is extended beyond 8 hours. HPLC analysis showed that malvidin-3-O-glucoside and epigallocatechin was respectively the two major anthocyanins and tannins in musts. Biological activities analyses of musts showed that higher antidiabetic and anti-inflammatory activities were more correlated to the high anthocyanin and phenolic acid content.

Finally, PCA results indicated that the accumulation of phenolic compounds in grape berries is strongly affected by terroir factors and Syrah Florentine was the terroir presenting higher stilbene enriched wines. Moreover, temperature and length of maceration are parameters that must be adjusted to grape varieties and defined terroir.

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A-B-C

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PART 2- Vintage Effect

II.2.1. Introduction

Phenolic compounds play one of the most important roles in the quality of red grapes and wines. Most of the main sensory attributes such as color, body, color, mouthfeel, astringency and bitterness are directly associated with the composition of wine in phenolic compounds (Vidal et al., 2003). In addition these compounds have been reported to have multiple biological health-promoting properties (Soleas et al., 1997; Zern et al., 2005; Pelligrini et al., 1996; Jang et al., 1997; de la Torre et al., 2006). During red winemaking process, phenolic compounds are extracted from grape skins along maceration and transferred to the must. Numerous factors such as grape varieties, length of skin contact, temperature of maceration, vintage, stages of ripeness, climatic factors such as sunlight exposure and solar radiation and the presence of macerating enzymes have all been shown to affect the extraction of phenolic into the must (Canals et al., 2005; Gómez- plaza et al., 2001, Cohen et al., 2008). Among these factors; two oenological practices are widely used in winemaking industries which are the prefermentative heat maceration and the addition of enzymes (Baustita-Ortin et al., 2012; Netzel et al., 2003). Prefermentative maceration at high temperatures (between 65°C and 80°C) is employed to extract phenolic compounds, denature alteration enzymes and destruct vegetal aromas of grapes. Several papers have presented results related to the use of this technique in the vinification of grapes from different varieties (Moutounet et al., 2000; Morel-Salmi et al., 2006; Fulcrand et al., 2004). Moreover, the use of commercial macerating pectolytic enzymes in winemaking is a common and well-known practice. These preparations by hydrolyzing the polysaccharides structural of grape skin cell walls favors the extraction of phenolic and aroma compounds contained within the solid part of the grape, mainly in the pulp and in the skin and improve the clarification processes of the must (Bisson and Butzke 1996; Canal-Llaubères, 2002). However, the effect of the addition of enzymes on the phenolic content remains unclear because of some contradictory results in the literature. Some workers have reported in increase in the total phenol and anthocyanin levels (Pardo et al., 1999; Bautista-Ortin et al., 2005; Romero-Cascales et al., 2012), whereas others have reported a decrease in the anthocyanin levels (Kelebek et al., 2007; Borazan and Bozan 2013). Moreover, the effect of the enzymatic preparations is also conditioned by the structure and composition of the skin cell walls. This effect therefore can be very different, depending on the grape variety, because genetic factors regulate these features (Ortega-Regules et al., 2008). Although a large number of studies on the phenolic composition of red

wines and their relationship with winemaking technology have been established (Bautista-Ortín et al., 2007; Berger and Cottereau, 2000), the published studies concerning the change in the phenolic of red musts during the winemaking process are scarce in the literature. Thus, the objective of this study was to investigate the influence of pectolytic enzyme addition and prefermentative heat maceration at different temperatures (60°C and 70°C) on the phenolic content and biological activities of Syrah and Cabernet Sauvignon red musts from two consecutive vintages (2014 and 2015) grown at Lebanese wine region and to elucidate by means of statistical multivariate analyses (PCA) the vintage effects

II.2.2. Materials and methods

II.2.2.1. CHEMICALS AND STANDARDS (see II.1.2.1, p. 86)

II.2.2.2. SAMPLES

Red grapes of *Vitis vinifera* var. Syrah (Sy) and Cabernet Sauvignon (CS) were supplied by Chateau Saint Thomas (West Bekaa /Lebanon) from two consecutive vintages 2014 and 2015. Grapes were harvested in 2014 and 2015 at maturity. The physiological ripeness of the berry samples was assessed by measurement of °Brix and titratable acidity (g/l sulfuric acid). °Brix is directly related to the sugar content (g/l) and potential titratable alcohol (Vol %). In addition sugar concentration increased throughout the maturation time, whereas titratable acidity decreased. All these data confirm that the two successive vintages of Cabernet Sauvignon had the same ripening stage, whereas the 2015 Syrah vintage showed higher levels of maturity than the 2014 vintage.The physiochemical properties for the two grape varieties from the two vintages are given in Table II.2.1 (Brix= 21.2 and 22.4 g/l; titrable acidity = 4.4 and 3.6 g/l as sulfuric acid for Sy 2014 and 2015 respectively; Brix= 24.2 and 24.2 g/l and; titrable acidity = 3.7 and 3.6 g/l as sulfuric acid for CS 2014 and 2015 respectively). At last, Meteorological data (temperature and precipitation) were provided by LARI weather station in Hawsh-Ammik (the nearest station to chateau Saint Thomas), placed at GPS coordinate X= 35.784302 and Y= 33.714857. Averaged temperatures from May to September were set at 22.4°C for the two vintages, total precipitation for the 2014 and 2015 vintage were respectively 366.2 mm and 228.6 mm.

Table II.2.1: Parameters of the two grape Cultivars from the two vintages

Samples	°Brix	Sugar content (g/L)	Potential alcohol (Vol %)	Titratable acidity (g/L sulfuric acid)
Sy-2014	21.2	205.5	12.2	4.4
Sy-2015	22.4	221	13	3.6
CS-2014	24.2	236.6	14	3.7
CS-2015	24.2	238	14	3.6

II.2.2.3. STRAINS AND STORAGE CONDITIONS (see II.1.2.3. p. 87)

II.2.2.4. MACERATION AND FERMENTATION PROCEDURES AND SAMPLING

After reception of the grapes they were crushed and destemmed manually, damaged clusters were removed manually and sodium metabisulphite was added (5 g of $NaHSO_3$/100 kg). 2 kg lots of grapes were drawn into glass Erlenmeyer flasks of 2L and the pre-fermentative maceration was conducted at different temperatures (60°C, 70°C and 70°C + enzyme) for 24 hours. The macerations were monitored and the kinetic profile of the maceration was studied by taking samples at 0, 2, 4, 8 and 24 hours. Based on data collected from the maceration part of the 2014 vintage, temperatures of 10°C and 80°C were abandoned for the 2015 vintage and maceration time was fixed at 24 hours. In fact, results from the 2014 vintage showed that after 24 hours of maceration some tannin were degraded, as well as, temperature of 10°C did not show an important evolution of phenolic compounds over time while temperature of 80°C exhibited faster and higher decrease in anthocyanin concentrations at early stage of maceration. Classical winemaking process with and without added enzymes (maceration and fermentation occurs together at 25°C) of Syrah and Cabernet Sauvignon Saint Thomas were used as control. Musts issued from control were separately inoculated by *S. cerevisiae* Y yeast strain at an initial concentration of 3×10^6 cells/ml (Thoma counting chamber). The AF was followed until total or cessation of sugar consumption (< 2 g/l, DNS colorimetric method Miller, 1959) and finished after 10 days. Control samples were collected at the end of the alcoholic fermentation. At the latest 50 ml of each sample was collected and directly centrifuged for 5 minutes at 5000 rpm. The samples were stored at 0°C and analyses were done after maceration and fermentation times (control) were finished. Commercial pectolytic enzymes (5 g/100 kg grapes, LAFASE HE Grand

Cru, Laffort), were added 2 hours (at room temperature) prior to maceration at 70°C and at the beginning of maceration for the control with added enzymes (control 25°C + enzymes). All macerations were carried out in triplicate.

II.2.2.5. SPECTROPHOTOMETRIC DETERMINATIONS (see II.1.2.5. p. 88)

II.2.2.6. HPLC ANALYSIS OF PHENOLIC COMPOUNDS (see II.1.2.6. p. 89)

II.2.2.7. DETERMINATION OF BIOLOGICAL ACTIVITIES (see II.1.2.7. p. 89-93)

II.2.2.8. STATISTICAL DATA TREATMENT (see II.1.2.8. p. 93)

II.2.3. Results and Discussion

II.2.3.1. IMPACT OF MACERATION'S TIME AND TEMPERATURE ON POLYPHENOL COMPOSITION OF MUSTS

II.2.3.1.1. Total anthocyanins and tannins

Figure II.2.1-A and II.2.1-B showed respectively the evolution of total tannins versus total anthocyanins during the maceration of the 2014 and 2015 vintages of Syrah and Cabernet Sauvignon musts at different temperatures (60°C and 70°C) for 24 hours. By macerating at 60°C, the concentrations of anthocyanins and total tannins increase progressively to reach a maximum of anthocyanins after 24 hours for both grape varieties and vintages.

When comparing the 2 temperature of macerations, a more rapid increase in anthocyanin and tannin concentrations at 70°C was observed. Similarly, the maximums reached are greater. For the two grape varieties from the two consecutive vintages, tannins reach a maximum of 8434.32 mg/l and 11243.62 mg/l respectively for Syrah and Cabernet Sauvignon from 2014 vintage after 24 hours of maceration, while anthocyanins reach maximum concentrations of 666.46 mg/l and 925.75 mg/l respectively from 2014 vintage after 8 hours of maceration. Beyond these maximums, a decrease of 19% to 24% of total anthocyanins is observed for both grape varieties from the two vintages.

In comparison between vintages, 2014 vintage for Syrah and Cabernet Sauvignon showed the maximum values for anthocyanins ($[anthocyanins]_{Sy\text{-}2014}$ = 633.79 mg/l and $[anthocyanins]_{CS\text{-}2014}$ = 836.79 mg/l) and for tannins ($[tanins]_{Sy\text{-}2014}$ = 6037.40 mg/l and $[tanins]_{CS\text{-}2014}$= 8859.58 mg/l). Syrah and Cabernet Sauvignon musts of 2014 vintage showed respectively 1.5 to 2.8 times higher anthocyanin contents and 1.25 to 1.8 times higher tannin contents than the 2015 vintage after 24 hours of maceration. As seen previously (II.1.3.1.1. p. 94-95) total anthocyanin content increases with temperature and maceration time up to a certain limit while the extraction of tannins is progressive over time (Guerrero et al., 2009; Galvin 1993).

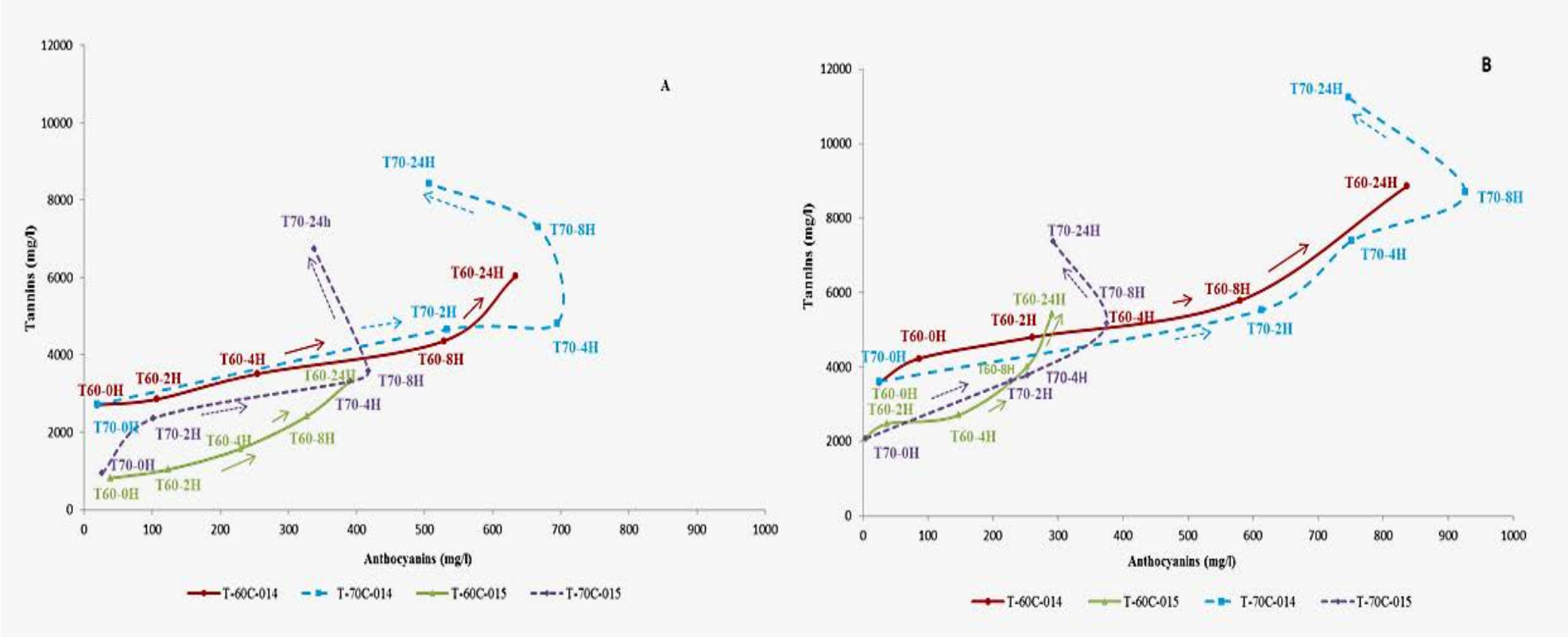

Figure II.2.1: Kinetics of tannins and anthocyanins extraction during the maceration of Syrah and Cabernet Sauvignon Saint Thomas grapes from the two consecutive vintages (2014 and 2015) in terms of time and temperature (A: Syrah musts, B: Cabernet Sauvignon musts, T-60C, T-70C: maceration temperatures respectively at 60°C and 70°C, example: T-60C-4H: maceration temperature at 60°C for 4 hours)

II.2.3.1.2. Total polyphenol, total polyphenol index and color intensity

Table II.2.2-a and II.2.2-b showed the evolution of total polyphenol, total polyphenol index and color intensity of Syrah and Cabernet Sauvignon musts from the two consecutive vintages (2014 and 2015) during pre-fermentation macerations at 60°C and 70°C compared to the control (classical vinification at 25°C).

Table II.2.2-a: Total polyphenol, total polyphenol index and color intensity of Syrah musts from the two consecutive vintages and the 2015 vintage of Syrah Saint Thomas control (25°C) in terms of time and temperature

		Sy maceration time (hours)										
			0		2		4		8		24	
		Control 25°C	ST-014	ST-015	ST-014	ST-015	ST-014	ST-015	ST-014	ST-015	ST-014	ST-015
60°C	CI	1.22 ± 0.01	0.34 ± 0.00^{a}	0.15 ± 0.03^{b}	0.611 ± 0.03^{a}	0.39 ± 0.01^{b}	0.99 ± 0.02^{a}	0.79 ± 0.03^{b}	1.34 ± 0.10^{a}	1.06 ± 0.06^{b}	1.53 ± 0.01^{a}	1.08 ± 0.06^{b}
	TPI	60.12 ± 2.57	16.27 ± 0.25^{a}	12.50 ± 0.20^{b}	21.97 ± 0.50^{a}	16.30 ± 0.56^{b}	29.97 ± 2.90^{a}	22.97 ± 1.25^{b}	35.17 ± 2.80^{a}	28.60 ± 0.91^{b}	52.93 ± 1.62^{a}	42.00 ± 2.19^{b}
	TP	2452.25 ± 46.19	441.67 ± 0.81^{a}	401.67 ± 2.87^{b}	680.00 ± 3.41^{a}	621.67 ± 5.77^{b}	873.30 ± 4.52^{a}	803.33 ± 79.73^{b}	1393.33 ± 2.51^{a}	1310.33 ± 18.92^{a}	2266.67 ± 5.12^{a}	2172.67 ± 28.43^{b}
70°C	CI	1.22 ± 0.01	0.34 ± 0.00^{a}	0.16 ± 0.02^{b}	1.30 ± 0.04^{a}	0.66 ± 0.02^{b}	1.39 ± 0.10^{a}	1.16 ± 0.06^{b}	1.59 ± 0.04^{a}	1.51 ± 0.09^{b}	1.60 ± 0.02^{a}	1.46 ± 0.09^{b}
	TPI	60.12 ± 2.57	16.70 ± 0.10^{a}	12.33 ± 0.21^{b}	37.43 ± 0.80^{a}	32.37 ± 0.46^{b}	49.93 ± 3.30^{a}	40.40 ± 0.26^{b}	56.00 ± 1.30^{a}	45.30 ± 0.62^{b}	73.73 ± 2.47^{a}	60.47 ± 1.97^{b}
	TP	2452.25 ± 46.19	440.00 ± 1.41^{a}	402.67 ± 12.58^{a}	1526.67 ± 1.92^{a}	1475.00 ± 13.23^{b}	2155.00 ± 2.74^{a}	2051.67 ± 2.88^{a}	2758.33 ± 1.30^{a}	2576.67 ± 5.77^{a}	3585.00 ± 1.97^{a}	3468.33 ± 2.88^{a}

Mean (n =3) ± SD. For each maceration time from the two consecutive vintages (2014 and 2015), different letters in the same row indicate significant difference at $p < 0.05$. CI, Color intensity; TPI, total phenolic index; TP, total phenolic; ST-014, Syrah Saint Thomas 2014; ST-015, Syrah Saint Thomas 2015

Table II.2.2-b: Total polyphenol, total polyphenol index and color intensity of Cabernet Sauvignon musts from the two consecutive vintages and the 2015 vintage of Cabernet Sauvignon Saint Thomas control (25°C) in terms of time and temperature

		CS maceration time (hours)										
			0		2		4		8		24	
		Control 25°C	CT-014	CT-015	CT-014	CT-015	CT-014	CT-015	CT-014	CT-015	CT-014	CT-015
60°C	CI	1.20 ± 0.01	0.21 ± 0.01^{a}	0.11 ± 0.00^{b}	0.31 ± 0.01^{a}	0.23 ± 0.01^{b}	0.41 ± 0.03^{a}	0.39 ± 0.01^{a}	0.73 ± 0.04^{a}	0.72 ± 0.02^{a}	1.46 ± 0.02^{a}	0.91 ± 0.03^{b}
	TPI	50.19 ± 0.04	12.37 ± 0.20^{a}	10.37 ± 0.38^{b}	13.67 ± 0.50^{a}	11.20 ± 0.70^{b}	17.47± 0.15^{a}	17.17 ± 0.15^{a}	26.23 ± 1.13^{a}	23.53 ± 0.66^{b}	44.00 ± 1.00^{a}	38.63 ± 0.51^{b}
	TP	2250.35 ± 5.77	616.67 ± 2.20^{a}	616.67 ± 2.60^{a}	678.33 ± 2.67^{b}	781.67 ± 1.92^{a}	815.00 ± 1.02^{b}	951.67 ± 0.63^{a}	1350.00 ± 0.10^{a}	1285.00 ± 1.62^{a}	2160.00 ± 2.32^{a}	2198.33 ± 0.32^{a}
70°C	CI	1.20 ± 0.01	0.20 ± 0.01^{a}	0.13 ± 0.00^{b}	0.70 ± 0.04^{a}	0.57 ± 0.04^{b}	1.017 ± 0.04^{a}	0.87 ± 0.01^{b}	1.59 ± 0.05^{a}	1.17 ± 0.02^{b}	1.49 ± 0.04^{a}	1.26 ± 0.01^{b}
	TPI	50.19 ± 0.04	12.77 ± 0.60^{a}	11.47 ± 0.30^{b}	27.30 ± 1.51^{a}	24.97 ± 0.76^{a}	31.40 ± 0.85^{a}	28.70 ± 0.35^{b}	45.63 ± 2.41^{a}	38.10 ± 0.37^{b}	62.73 ± 0.61^{a}	56.60 ± 2.07^{b}
	TP	2250.35 ± 5.77	601.67 ± 3.21^{b}	763.33 ± 2.33^{a}	1325.00 ± 2.24^{a}	1350.00 ± 1.41^{a}	1745.00 ± 1.54^{a}	1806.67 ± 0.92^{a}	2520.00 ± 2.49^{a}	1883.33 ± 1.77^{b}	3766.67 ± 1.51^{a}	3180.00 ± 1.02^{b}

Mean (n =3) ± SD. For each maceration time from the two consecutive vintages (2014 and 2015), different letters in the same row indicate significant difference at $p < 0.05$. CI, Color intensity; TPI, total phenolic index; TP, total phenolic; CT-014, Cabernet Sauvignon Saint Thomas 2014; CT-015, Cabernet Sauvignon Saint Thomas 2015

According to the results obtained from Table II.2.2-a and II.2.2-b, color intensity increases gradually at 60°C to reach its maximum at 24 hours ($CI_{Sy\text{-}014}$ =1.53 and $CI_{Sy\text{-}015}$ = 1.08; $CI_{CS\text{-}014}$ =1.46 and $CI_{CS\text{-}014}$ =0.91). On the opposite, a high increase in color intensity was observed at 70°C this maximum was reached after 8h for 2014 vintage of Syrah and Cabernet sauvignon ($CI_{Sy\text{-}CS\text{-}014}$ = 1.59) and 2015 vintage of Syrah ($CI_{Sy\text{-}015}$ = 1.51) and after 24h for 2015 vintage of Cabernet Sauvignon($CI_{CS\text{-}015}$= 1.26). Thus, color intensity showed the same trends than total anthocyanin content (Figure II.2.1-A and II.2.1-B). In fact their higher anthocyanin richness during the length of maceration increased the percentage of red (A520 nm) and yellow (A420 nm) excepting for 2015 vintage of CS at temperature of 70°C for which the lower values of anthocyanins (Figue II.2.1-B) were associated with the higher values of CI. This

can be explained as mentioned before (II.1.3.1.1. p. 94-95) by the formation of new compound due to copigmentation and condensations reactions (Galvin 1993). So after 24 hours of maceration 2014 vintage for the two grape musts showed the highest values of CI than 2015 vintage. Values were 1.41 and 1.10 times higher for Syrah 2014 respectively at temperature of 60°C and 70°C than Syrah 2015 and 1.60 and 1.18 times higher for CS respectively at temperature of 60°C and 70°C than 2015 vintage of CS.

The total polyphenols are characterized qualitatively by the total polyphenols index (TPI) and quantitatively by the analysis of the total polyphenol (TP) by the Folin-Ciocalteu method. The results showed an increase in TPI with temperature and over time. In fact when temperatures increase, the extraction of the polyphenols is more facilitated by the weakness of the cell membranes which results into an increase in the extraction of polyphenols in the must. After 24 hours of maceration, TPI was 52.93 (Sy-014); 42.00 (Sy-015); 44.00 (CS-014) and 38.63 (CS-015) at 60°C and 73.73 (Sy-014); 60.47 (Sy-015), 62.73 (CS-014) and 56.60 (CS-015) at 70°C. Cabernet Sauvignon and Syrah musts of 2014 vintage showed an average TPI value of 1.18 times higher than 2015 vintage at temperatures of 60°C and 70°C.

Concerning total polyphenols and during the maceration at 60°C, the maximum extraction is reached at 24 hours. The maximum concentrations obtained from Syrah and Cabernet Sauvignon musts are respectively 2266.67 (Sy-2014) and 2198.33 (CS-2015) mg/l GAE. At 70°C, a more rapid increase in total polyphenols was observed with higher maximum concentrations compared to 60°C. The maximum extraction is 3585.00 and 3766.67 mg/l GAE respectively for Syrah and Cabernet Sauvignon musts. As TPI, 2014 vintage of the two grape musts revealed higher concentrations of total polyphenols than 2015 vintage.

In other hand, the control from the two grape varieties indicated higher values of CI, TPI and TP than pre-macerated must at 60°C and lower values than pre-macerated must at 70°C after 24 hours (Tble II.2.2-a and II.2.2-b).

II.2.3.1.3. Anthocyanins profile

The evolution of HPLC individual anthocyanins during maceration of Syrah and Cabernet Sauvignon musts from the two consecutive vintages at different temperatures (60°C and 70°C) for 24 hours compared to the control (25°C) is shown in Table (II.2.3-a, II.2.3-b). During the maceration of grape musts at 60°C, malvidin-3-O-glucoside remains the most represented compound with a maximum concentration of 85.39 mg/l and 53.42mg/l respectively for Syrah 2014 and 2015 at 24 hours, and 149.81 mg/l and 95.82 mg/l respectively for Cabernet Sauvignon 2014 and 2015 after 24h. Delphinidin-3-O-glucoside and peonidin-3-O-glucoside reached their maximums of 11.74 mg/l (CS-2014) and 12.46 mg/l (Sy-2015) respectively after 24 hours. The evolution of cyanidin-3-O-glucoside over time remains very low. At 70°C, a marked improvement in anthocyanin extraction was observed. Maximum extraction is reached more rapidly at 4 hours for cyanidin-3-O-glucoside ($[cy]_{Sy-014}$ =1.84 mg/l; $[cy]_{CS-014}$ = 2.10 mg/l; $[cy]_{CS-015}$ = 1.64 mg/l and after 8 hours for Sy-2015 (4.88mg/l); after 8 hours for peonidin-3-O-glucoside ($[Pn]_{Sy-2015}$ = 16.36 mg/l; $[Pn]_{CS-2014}$ = 5.70 mg/l ; $[Pn]_{CS-2015}$ = 6.79 mg/l and 4 h for Sy-2014(10.46 mg/l) and 4 hours for malvidin-3-O-glucoside for Syrah musts ($[Mv]_{Sy-2014}$= 84.77mg/l ; $[Mv]_{Sy-2015}$ = 88.24 mg/l) and 8 h for CS musts ($[Mv]_{CS-2014}$= 151.01mg/l ; $[Mv]_{CS-2015}$ = 85.62 mg/l). The prolongation of the maceration causes degradation of the anthocyanidic compounds under the effect of the heat reaching 50% on certain compounds. With few exceptions, 2014 vintage from the two different musts showed significantly higher anthocyanins profiles than 2015. Syrah control showed higher individual monomeric anthocyanins than syrah musts from the two vintages macerated at temperatures of 60°C and 70°C after 24 hours, whereas CS control demonstrated values 2.45 and 1.23 times higher respectively for Dp and Cy than CS-2015 macerated at 60°C after 24 hours and 2.04; 1.35 and 1.22 times higher respectively for Dp; Cy and Mv than CS-2015 macerated at 70°C after 24 hours.

The higher amounts of anthocyanins monomers in control samples are due to the absence of high temperatures and the presence of ethanol which facilitates the diffusion of phenolic compounds from solid parts of the grapes to the must.

Table II.2.3-a: Anthocyanins profile (mg/l) of Syrah musts from the two consecutive vintages and the 2015 vintage of Syrah control (25°C) in terms of time and temperature

		Sy maceration time (hours)										
			0		2		4		8		24	
		Contol 25°C	ST-014	ST-015	ST-014	ST-015	ST-014	ST-015	ST-014	ST-015	ST-014	ST-015
60°C	Dp	6.00 ± 0.18	n.d	1.71 ± 0.13^{a}	0.86 ± 0.01^{b}	1.94 ± 0.02^{a}	0.85 ± 0.00^{b}	1.95 ± 0.00^{a}	1.59 ± 0.19^{b}	1.98 ± 0.03^{a}	6.150 ± 0.19^{a}	2.01 ± 0.01^{b}
	Cy	3.12 ± 0.04	n.d	1.62 ± 0.065^{a}	n.d	1.77 ± 0.09^{a}	n.d	3.54 ± 0.04^{a}	1.36 ± 0.04^{b}	3.27 ± 0.01^{a}	1.62 ± 0.01^{b}	2.23 ± 0.06^{a}
	Pn	6.10 ± 0.13	n.d	3.21 ± 0.06^{a}	0.98 ± 0.01^{b}	6.15 ± 0.45^{a}	1.85 ± 0.03^{b}	8.32 ± 0.54^{a}	6.06 ± 0.02^{b}	10.07 ± 0.58^{a}	10.97 ± 0.01^{b}	12.46 ± 0.79^{a}
	MV	65.35 ± 0.51	n.d	12.76 ± 0.03^{a}	9.79 ± 0.00^{b}	14.61 ± 0.66^{a}	15.16 ± 0.01^{b}	20.11 ± 0.66^{a}	40.56 ± 0.04^{b}	41.91 ± 2.24^{a}	85.39 ± 0.03^{a}	53.42 ± 1.03^{b}
70°C	Dp	6.00 ± 0.18	n.d	1.62 ± 0.03^{a}	4.17 ± 0.01^{a}	1.82 ± 0.05^{b}	4.43 ± 0.04^{a}	1.87 ± 0.04^{b}	6.34 ± 0.02^{a}	2.33 ± 0.06^{b}	5.53 ± 0.00^{a}	2.14 ± 0.01^{b}
	Cy	3.12 ± 0.04	n.d	1.32 ± 0.04^{a}	1.76 ± 0.00^{b}	2.65 ± 0.00^{a}	1.84 ± 0.02^{b}	3.19 ± 0.07^{a}	1.56 ± 0.03^{b}	4.88 ± 0.05^{a}	1.37 ± 0.01^{b}	2.71 ± 0.05^{a}
	Pn	6.10 ± 0.13	n.d	3.12 ± 0.01^{a}	9.44 ± 0.03^{b}	11.01 ± 0.22^{a}	10.46 ± 0.03^{b}	13.55 ± 0.32^{a}	7.16 ± 0.03^{b}	16.36 ± 0.35^{a}	10.77 ± 0.03^{b}	12.51 ± 0.25^{a}
	MV	65.35 ± 0.51	n.d	7.87 ± 0.04^{a}	56.14 ± 0.04^{a}	55.41 ± 0.66^{a}	84.77 ± 0.03^{b}	88.24 ± 1.35^{a}	42.05 ± 0.02^{b}	44.6 ± 1.60^{a}	28.73 ± 0.00^{a}	10.65 ± 2.05^{b}

Mean (n =3) ± SD. For each maceration time from the two consecutive vintages (2014 and 2015), different letters in the same row indicate significant difference at $p < 0.05$. Dp, delphinidin-3-O-glucoside; Cy, cyanidin-3-O-glucoside; Pn, peonidin-3-O-glucoside; Mv, malvidin-3-O-glucoside; ST-014, Syrah Saint Thomas 2014; ST-015, Syrah Saint Thomas 2015; n.d., not detected values

Table II.2.3-b: Anthocyanins profile (mg/l) of Cabernet Sauvignon musts from the two consecutive vintages and the 2015 vintage of Cabernet Sauvignon control (25°C) in terms of time and temperature

		CS maceration time (hours)										
			0		2		4		8		24	
		Control 25°C	CS-014	CS-015	CS-014	CS-015	CS-014	CS-015	CS-014	CS-015	CS-014	CS-015
60°C	Dp	4.63 ± 0.30	n.d	n.d	n.d	n.d	0.8 ± 0.04^{b}	1.55 ± 0.01^{a}	4.24 ± 0.04^{a}	2.17 ± 0.05^{b}	11.74 ± 0.03^{a}	1.89 ± 0.01^{b}
	Cy	1.91 ± 0.00	n.d	1.28 ± 0.00^{a}	n.d	1.33 ± 0.03^{a}	0.00 ± 0.00^{b}	1.44 ± 0.04^{a}	1.10 ± 0.02^{b}	1.55 ± 0.01^{a}	2.42 ± 0.02^{a}	1.55 ± 0.02^{b}
	Pn	2.92 ± 0.01	n.d	1.14 ± 0.00^{a}	0.08 ± 0.02^{b}	1.18 ± 0.01^{a}	0.65 ± 0.05^{b}	3.18 ± 0.06^{a}	3.20 ± 0.032^{b}	5.16 ± 0.00^{a}	4.80 ± 0.04^{b}	6.31 ± 0.14^{a}
	MV	66.35 ± 1.98	1.20 ± 0.05^{b}	10.53 ± 0.24^{a}	16.25 ± 0.04^{b}	17.58 ± 0.44^{a}	29.04 ± 0.05^{b}	54.87 ± 0.63^{a}	85.17 ± 0.05^{a}	83.27 ± 0.71^{b}	149.81 ± 0.02^{a}	95.82 ± 1.11^{b}
70°C	Dp	4.63 ± 0.30	n.d	n.d	4.24 ± 0.01^{a}	n.d	7.36 ± 0.01^{a}	1.63 ± 0.01^{b}	14.33 ± 0.02^{a}	1.88 ± 0.00^{b}	18.26 ± 0.03^{a}	2.27 ± 0.01^{b}
	Cy	1.91 ± 0.00	n.d	1.26 ± 0.04^{a}	1.23 ± 0.01^{b}	1.60 ± 0.06^{a}	1.62 ± 0.10^{a}	1.65 ± 0.06^{a}	2.10 ± 0.05^{a}	1.64 ± 0.04^{b}	1.54 ± 0.02^{a}	1.41 ± 0.04^{b}
	Pn	2.92 ± 0.01	n.d	1.14 ± 0.01^{a}	3.41 ± 0.01^{b}	4.84 ± 0.11^{a}	4.29 ± 0.02^{b}	5.54 ± 0.01^{a}	5.70 ± 0.03^{b}	6.79 ± 0.17^{a}	3.59 ± 0.05^{b}	4.87 ± 0.04^{a}
	MV	66.35 ± 1.98	1.24 ± 0.05^{b}	10.99 ± 0.57^{a}	73.63 ± 0.03^{a}	60.79 ± 0.92^{b}	121.90 ± 0.01^{a}	77.14 ± 1.88^{b}	151.01 ± 0.02^{a}	85.62 ± 2.19^{b}	82.32 ± 0.05^{a}	54.54 ± 1.41^{b}

Mean (n =3) ± SD. For each maceration time from the two consecutive vintages (2014 and 2015), different letters in the same row indicate significant difference at $p < 0.05$. Dp, delphinidin-3-O-glucoside; Cy, cyaniding-3-O-glucoside; Pn, peonidin-3-O-glucoside; Mv, malvidin-3-O-glucoside; CT-014, Cabernet Sauvignon Saint Thomas 2014; CT-015, Cabernet Sauvignon Saint Thomas 2015; n.d., not detected values

II.2.3.1.4. Flavan-3-ols and non-flavonoids profile

The evolution of monomeric and dimeric tannins, phenolic acids and stilbenes during the maceration of Syrah and Cabernet Sauvignon musts from the two consecutive vintages (2014 and 2015) at different temperatures (60°C and 70°C) for 24 hours compared to the control (Sy and CS-25°C- 2015) were shown in Tables II.2.4-a and II.2.4-b.

Concerning monomeric and dimeric tannins, their extraction is favored by higher temperatures (70°C, Table II.2.4-a and II.2.4-b). In terms of concentration, epigallocatechin is the most represented monomer of flavan-3-ols. At 60°C, the maximum extraction of catechin, epicatechin, epigallocatechin and epicatechin gallate was obtained after 24 hours of maceration and maximum concentrations are respectively 56.82 mg/l (Sy-2015); 101.06 mg/l (Sy-2015); 216.95 mg/l (Sy-2014) and 18.22 mg/l (CS-2014). The maceration at 70°C improves the extraction of tannins whose maximum values are multiplied by an average factor of 1.48; 1.41; 1.52 and 2.20 respectively for catechin, epicatechin, epicatechin gallate and epigallocatechin for syrah musts and an average factor of 2.17; 2.39; 2.00 and 4.13 respectively for catechin, epicatechin, epicatechin gallate and epigallocatechin for cabernet sauvignon musts. With few exceptions, 2015 vintage of Sy and CS musts showed significantly higher values of catechin and epicatechin for the different maceration temperatures than for the 2014 vintage of the two different grape musts. Total monomeric tannins for syrah control were on average 1.33 and 2.54 times lower than syrah musts macerated respectively at temperatures of 60°C and 70°C. Whereas, CS control had an average value of 1.58 times higher for total monomeric tannins than CS macerated at 60°C and an average value of 2.09 times lower than CS musts macerated at 70°C from the two vintages.

For the dimeric tannins, the maceration at 70°C increases the concentration of procyanidin B1 and B2 respectively by 58.00% and 7.56% for Sy-2014; 31.67% and 16.38% for Sy-2015; 52.03% and 49.52% for CS-2014 and 47.34% and 29.15% for CS-2015. Unlike anthocyanins, tannins appear to resist thermal degradation. In fact, longer maceration times seem to favor the extraction of tannins because the release of these compounds occurs from the grape skins and seeds (Guerrero et al., 2009). Among grape varieties and vintages, 2014 vintage of CS showed (Table II.2.4-b) significantly higher values of dimeric tannins (Pro B1 and B2) than for 2014, whereas, 2015 vintage of syrah musts revealed significantly higher values of Procyanidin B2 than for Syrah 2014. CS control showed higher total dimeric tannins than CS macerated at 60°C

and lower values than CS macerated at 70°C, while Sy control showed lower total dimeric tannins than syrah musts macerated at temperatures of 60°C and 70°C (except for Sy-60°C-2015). Concerning the phenolic acids, the highest temperature increases the levels of the compounds obtained after 24 hours of maceration with some exceptions as for example gallic acid which showed high heat sensitivity as in the case of Sy-2014, where gallic aicd is no longer detected by HPLC after 8 hours of macerations. Thus, gallic acid, ferulic acid and caffeic acid had higher maximums at 70°C (34.98 mg/l (CS-2015), 80.20 mg/l (Sy-2015) and 12.87mg/l (Sy-2014) respectively) In addition, 2014 vintage of the different musts (Table II.2.4-a and II.2.4-b) showed significantly higher values of caffeic acid whereas 2015 vintage showed significantly higher values of gallic and ferulic for the different temperatures and grape varieties. Sy control showed higher phenolic acids values than Sy-60°C from the two vintages and Sy-70°C-2014, whereas CS control exhibited higher phenolic acids values than CS musts macerated at temperatures of 60°C and 70°C from both vintages. Eventually, regarding stilbenes, the highest level of resveratrol is obtained by macerating at 70°C for 24 hours (15.35 mg/l, CS-2014) without any detection of degradation. 2014 vintage of the different musts indicated significantly higher values of resveratrol which is on average value almost twice higher than for the 2015 musts. Sy and CS 2014 vintage macerated at 70°C showed values 1.34 and 2.15 times higher respectively than Sy and CS control. Our results showed, as seen previously (II.1.3.1.4. p. 108) that epigallocatechin which is only found in the skin of grape berries was the most represented monomer of flavan-3-ols which indicates that the skin tannins are extracted preferentially during the first hours of maceration while the release of flavan-3-ols from the seeds requires longer maceration times or the presence of ethanol (Guerrero et al. 2009)

Table II.2.4-a: Flavan-3-ols and non-flavonoids profile (mg/l) of Syrah musts from the two consecutive vintages and the 2015 vintage of Syrah control (25°C) in terms of time and temperature

		Sy maceration time (hours)										
			0		2		4		8		24	
		Control 25°C	ST-014	ST-015	ST-014	ST-015	ST-014	ST-015	ST-014	ST-015	ST-014	ST-015
60°C	Cat	53.00 ± 0.34	8.68 ± 0.17^{a}	6.34 ± 0.18^{b}	12.09 ± 0.26^{a}	13.1 ± 0.76^{a}	9.54 ± 0.09^{b}	24.04 ± 0.92^{a}	18.81 ± 0.64^{b}	42.34 ± 0.90^{a}	32.21 ± 0.31^{b}	56.82 ± 1.08^{a}
	Epi	90.22 ± 0.76	2.07 ± 0.07^{b}	9.27 ± 0.23^{a}	7.48 ± 0.08^{b}	20.07 ± 0.43^{a}	7.87 ± 0.27^{b}	37.03 ± 1.25^{a}	21.45 ± 0.51^{b}	64.47 ± 2.06^{a}	41.32 ± 1.20^{b}	101.06 ± 1.29^{a}
	Epig	22.13 ± 0.89	1.95 ± 0.02^{b}	4.49 ± 0.25^{a}	5.33 ± 0.02^{b}	7.48 ± 0.53^{a}	10.07 ± 0.24^{a}	10.14 ± 0.10^{a}	14.79 ± 0.32^{a}	11.75 ± 1.00^{b}	12.35 ± 0.39^{a}	12.53 ± 0.49^{a}
	EpiG	72.32 ± 0.29	33.40 ± 0.15^{b}	44.66 ± 1.93^{a}	76.61 ± 0.12^{a}	37.58 ± 0.13^{b}	62.76 ± 0.14^{a}	43.26 ± 1.62^{b}	209.02 ± 0.41^{a}	77.61 ± 1.55^{b}	216.95 ± 0.41^{a}	160.82 ± 0.27^{b}
	Σmonomerics	237.67 ± 0.57	46.10 ± 0.10^{b}	64.76 ± 0.65^{a}	101.51 ± 0.48^{a}	78.23 ± 0.46^{b}	90.24 ± 0.18^{b}	114.47 ± 0.97^{a}	264.07 ± 0.47^{a}	196.17 ± 1.38^{b}	302.83 ± 0.58^{b}	331.23 ± 0.78^{a}
	Pro B1	110.05 ± 0.28	4.39 ± 0.06^{b}	9.04 ± 0.00^{a}	7.26 ± 0.24^{b}	24.35 ± 1.01^{a}	16.37 ± 0.50^{b}	32.18 ± 0.63^{a}	43.51 ± 1.38^{a}	28.49 ± 0.22^{b}	225.89 ± 0.53^{a}	32.97 ± 0.81^{b}
	Pro B2	115.32 ± 0.32	6.34 ± 0.23^{b}	24.75 ± 0.40^{a}	14.24 ± 0.30^{b}	62.47 ± 0.36^{a}	13.37 ± 0.40^{b}	63.42 ± 0.13^{a}	35.03 ± 1.75^{b}	85.31 ± 0.39^{a}	82.24 ± 2.96^{b}	101.89 ± 1.56^{a}
	Σdimerics	225.37 ± 0.30	10.73 ± 0.14^{b}	33.79 ± 0.40^{a}	21.50 ± 0.27^{b}	86.82 ± 0.68^{a}	29.74 ± 0.45^{b}	95.60 ± 0.38^{a}	78.54 ± 1.56^{b}	113.80 ± 0.30^{a}	308.13 ± 1.74^{a}	134.86 ± 1.18^{b}
	G.A	25.10 ± 0.10	1.99 ± 0.04^{b}	5.85 ± 0.16^{a}	1.97 ± 0.00^{b}	6.12 ± 0.39^{a}	2.46 ± 0.09^{b}	6.06 ± 0.02^{a}	n.d	5.96 ± 0.04^{a}	n.d	12.16 ± 0.68^{a}
	F.A	60.22 ± 0.40	1.98 ± 0.06^{b}	10.69 ± 1.19^{a}	4.46 ± 0.01^{b}	17.075 ± 0.21^{a}	8.85 ± 0.12^{b}	38.83 ± 1.04^{a}	12.36 ± 0.47^{b}	54.4 ± 1.78^{a}	18.66 ± 0.43^{b}	57.11 ± 1.14^{a}
	C.A	25.08 ± 0.15	1.85 ± 0.01^{b}	3.48 ± 0.29^{a}	2.92 ± 0.02^{b}	3.49 ± 0.27^{a}	3.39 ± 0.07^{a}	3.21 ± 0.17^{a}	4.08 ± 0.02^{a}	3.85 ± 0.03^{b}	5.73 ± 0.20^{a}	4.65 ± 0.10^{b}
	Σ phenolic acids	110.40 ± 0.22	5.82 ± 0.04^{b}	20.02 ± 0.55^{a}	9.35 ± 0.01^{b}	26.69 ± 0.29^{a}	14.70 ± 0.28^{b}	48.10 ± 0.41^{a}	16.44 ± 0.24^{b}	64.21 ± 0.61^{a}	24.39 ± 0.31^{b}	73.92 ± 0.64^{a}
	Res	7.14 ± 0.00	1.47 ± 0.00^{b}	2.37 ± 0.10^{a}	1.60 ± 0.00^{b}	2.72 ± 0.14^{a}	2.67 ± 0.10^{b}	3.18 ± 0.01^{a}	4.32 ± 0.60^{a}	3.42 ± 0.07^{a}	3.34 ± 0.10^{a}	3.40 ± 0.07^{a}
70°C	Cat	53.00 ± 0.34	9.53 ± 0.15^{b}	19.73 ± 0.53^{a}	20.87 ± 0.69^{b}	33.03 ± 0.92^{a}	19.42 ± 0.53^{a}	16.55 ± 2.31^{a}	27.98 ± 0.67^{b}	43.86 ± 0.72^{a}	34.12 ± 1.47^{b}	108.72 ± 0.64^{a}
	Epi	90.22 ± 0.76	2.23 ± 0.01^{b}	17.80 ± 1.00^{a}	20.56 ± 0.68^{b}	29.48 ± 1.05^{a}	22.07 ± 0.76^{b}	43.66 ± 2.91^{a}	39.41 ± 0.38^{b}	56.54 ± 1.04^{a}	61.14 ± 1.44^{b}	135.33 ± 2.72^{a}
	Epig	22.13 ± 0.89	2.25 ± 0.02^{a}	5.87 ± 0.33^{a}	9.92 ± 0.28^{a}	6.38 ± 0.59^{b}	12.56 ± 0.27^{a}	9.69 ± 0.12^{b}	14.60 ± 0.51^{a}	13.94 ± 0.79^{a}	19.52 ± 0.29^{a}	18.36 ± 2.22^{b}
	EpiG	72.32 ± 0.29	42.71 ± 2.11^{a}	41.39 ± 0.66^{a}	150.79 ± 0.50^{a}	33.87 ± 2.50^{b}	184.22 ± 1.45^{a}	54.63 ± 0.32^{b}	596.292 ± 1.37	129.34 ± 0.85^{b}	488.66 ± 1.90^{a}	347.74 ± 2.47^{b}
	Σmonomerics	237.67 ± 0.57	56.72 ± 0.57^{b}	84.79 ± 0.63^{a}	202.14 ± 0.54^{a}	102.76 ± 1.26^{b}	238.27 ± 3.01^{a}	124.53 ± 1.41^{b}	678.28 ± 0.73^{a}	243.68 ± 0.85^{b}	603.44 ± 1.27^{b}	610.15 ± 2.01^{a}
	Pro B1	110.05 ± 0.28	47.79 ± 0.09^{a}	9.33 ± 0.51^{b}	163.55 ± 2.70^{a}	21.73 ± 1.89^{b}	162.54 ± 0.90^{a}	30.68 ± 0.37^{b}	215.11 ± 0.23^{a}	101.53 ± 1.23^{b}	265.03 ± 0.05^{a}	161.05 ± 1.14^{b}
	Pro B2	115.32 ± 0.32	7.42 ± 0.15^{b}	24.85 ± 1.14^{a}	28.90 ± 0.47^{b}	41.16 ± 1.60^{a}	48.11 ± 1.99^{b}	72.58 ± 2.03^{a}	80.02 ± 1.33^{b}	103.61 ± 0.75^{a}	124.04 ± 1.34^{b}	134.21 ± 0.64^{a}
	Σdimerics	225.37 ± 0.30	55.21 ± 0.12^{a}	34.18 ± 0.82^{b}	192.45 ± 1.58^{a}	62.89 ± 1.74^{b}	210.65 ± 1.46^{a}	103.26 ± 1.20^{b}	295.13 ± 0.78^{a}	205.14 ± 0.99^{b}	389.07 ± 0.69^{a}	295.26 ± 0.89^{b}
	G.A	25.10 ± 0.10	2.01 ± 0.05^{b}	5.71 ± 0.50^{a}	2.26 ± 0.08^{b}	5.52 ± 0.66^{a}	4.95 ± 0.04^{b}	6.43 ± 0.18^{a}	n.d	6.01 ± 0.44^{a}	n.d	29.45 ± 0.22^{a}
	F.A	60.22 ± 0.40	2.09 ± 0.05^{b}	7.27 ± 0.02^{a}	15.95 ± 0.71^{b}	24.81 ± 0.43^{a}	15.03 ± 0.61^{b}	26.31 ± 0.61^{a}	15.85 ± 0.46^{b}	61.95 ± 1.41^{a}	16.33 ± 0.04^{b}	80.20 ± 2.75^{a}
	C.A	25.08 ± 0.15	1.94 ± 0.06^{b}	3.56 ± 0.31^{a}	3.33 ± 0.04^{b}	3.69 ± 0.08^{a}	3.42 ± 0.14^{b}	4.19 ± 0.00^{a}	8.72 ± 0.15^{a}	6.32 ± 0.09^{b}	12.87 ± 0.08^{a}	9.45 ± 0.20^{b}
	Σ phenolic acids	110.40 ± 0.22	6.04 ± 0.05^{b}	16.54 ± 0.28^{a}	21.54 ± 0.28^{b}	34.02 ± 0.39^{a}	23.40 ± 0.26^{b}	36.93 ± 0.26^{a}	24.57 ± 0.30^{b}	74.28 ± 0.65^{a}	29.20 ± 0.06^{b}	119.10 ± 1.06^{a}
	Res	7.14 ± 0.00	1.48 ± 0.04^{b}	2.51 ± 0.13^{a}	4.74 ± 0.22^{a}	2.80 ± 0.08^{b}	5.28 ± 0.10^{a}	4.48 ± 0.28^{b}	6.64 ± 0.23^{a}	4.12 ± 0.20^{b}	9.57 ± 0.38^{a}	6.84 ± 0.03^{b}

Mean (n =3) ± SD. For each maceration time from the two vintages, different letters in the same row indicate significant difference at $p < 0.05$Cat, catechin; Epi, epicatechin; Epig, epicatechin gallte; EpiG, epigallocatechin; Pro B1, procyanidin B1; Pro B2, procyanidin B2; G.A., gallic acid; F.A., ferulic acid; C.A., caffeic acid; Res, resveratrol; ST-014, Syrah Saint Thomas 2014; ST-015, Syrah Saint Thomas 2015; n.d., not detected values

Table II.2.4-b: Flavan-3-ols and non-flavonoids profile (mg/l) of Cabernet Sauvignon musts from the two consecutive vintages and the 2015 vintage of Cabernet Sauvignon control (25°C) in terms of time and temperature

		CS maceration time (hours)										
			0		2		4		8		24	
		Control 25°C	CS-014	CS-015	CS-014	CS-015	CS-014	CS-015	CS-014	CS-015	CS-014	CS-015
60°C	Cat	46.02 ± 0.10	8.23 ± 0.01^{a}	4.81 ± 0.13^{b}	7.08 ± 0.01^{b}	10.07 ± 0.07^{a}	16.44 ± 0.02^{a}	12.35 ± 0.13^{b}	22.44 ± 0.04^{a}	17.10 ± 0.51^{b}	30.89 ± 0.01^{b}	35.28 ± 0.39^{a}
	Epi	78.25 ± 1.01	3.46 ± 0.03^{b}	6.07 ± 0.03^{a}	7.15 ± 0.01^{b}	12.11 ± 0.16^{a}	11.20 ± 0.01^{b}	16.30 ± 0.13^{a}	13.45 ± 0.02^{b}	17.22 ± 0.86^{a}	33.67 ± 0.01^{a}	37.28 ± 0.21^{a}
	Epig	29.50 ± 0.36	1.86 ± 0.01^{b}	2.37 ± 0.03^{a}	4.69 ± 0.05^{a}	3.79 ± 0.05^{b}	7.62 ± 0.02^{a}	4.17 ± 0.04^{b}	9.74 ± 0.01^{a}	6.17 ± 0.02^{b}	18.22 ± 0.02^{a}	10.26 ± 0.02^{b}
	EpiG	140.20 ± 0.04	27.59 ± 0.04^{a}	23.63 ± 0.35^{b}	51.6 ± 0.01^{a}	32.02 ± 0.41^{b}	62.24 ± 0.01^{a}	45.19 ± 0.75^{b}	94.75 ± 0.03^{a}	87.83 ± 1.34^{b}	107.57 ± 0.03^{a}	97.59 ± 1.12^{b}
	Σmonomerics	293.97 ± 0.38	41.14 ± 0.02^{a}	36.88 ± 0.13^{b}	70.52 ± 0.02^{a}	57.99 ± 0.17^{b}	97.50 ± 0.01^{a}	78.01 ± 0.26^{b}	140.38 ± 0.02^{a}	128.32 ± 0.68^{b}	190.35 ± 0.02^{a}	180.41 ± 0.43^{b}
	Pro B1	134.10 ± 1.15	6.26 ± 0.01^{a}	6.49 ± 0.17^{a}	9.16 ± 0.01^{b}	22.18 ± 0.64^{a}	12.42 ± 0.05^{b}	24.49 ± 0.15^{a}	21.59 ± 0.05^{b}	47.81 ± 1.03^{a}	112.76 ± 0.03^{a}	107.50 ± 1.73^{a}
	Pro B2	96.45 ± 1.05	12.09 ± 0.04^{a}	4.86 ± 0.01^{b}	17.29 ± 0.05^{b}	22.69 ± 0.03^{a}	22.13 ± 0.05^{b}	27.65 ± 0.16^{a}	36.37 ± 0.02^{a}	31.44 ± 1.87^{b}	66.84 ± 0.01^{a}	60.18 ± 2.15^{b}
	Σdimerics	230.55 ± 1.10	18.35 ± 0.02^{a}	11.35 ± 0.09^{b}	26.45 ± 0.03^{b}	44.87 ± 0.33^{a}	34.55 ± 0.05^{b}	52.14 ± 0.15^{a}	57.96 ± 0.03^{b}	79.25 ± 1.45^{a}	179.60 ± 0.02^{a}	167.68 ± 1.94^{b}
	G.A	22.42 ± 0.17	2.08 ± 0.05^{a}	1.73 ± 0.08^{b}	2.07 ± 0.01^{a}	2.50 ± 0.07^{b}	2.34 ± 0.03^{b}	5.32 ± 0.02^{a}	3.26 ± 0.01^{b}	7.62 ± 0.20^{a}	3.56 ± 0.01^{b}	16.67 ± 0.07^{a}
	F.A	20.15 ± 0.14	2.27 ± 0.03^{b}	3.71 ± 0.25^{a}	3.02 ± 0.05^{b}	6.04 ± 0.03^{a}	2.72 ± 0.01^{b}	11.33 ± 0.06^{a}	7.49 ± 0.01^{b}	19.73 ± 0.23^{a}	14.54 ± 0.01^{b}	25.39 ± 0.53^{a}
	C.A	2.79 ± 0.09	1.84 ± 0.01^{b}	2.13 ± 0.11^{a}	2.07 ± 0.05^{a}	2.39 ± 0.34^{a}	2.42 ± 0.03^{a}	2.34 ± 0.04^{a}	5.12 ± 0.01^{a}	2.74 ± 0.11^{b}	9.19 ± 0.01^{a}	4.43 ± 0.00^{b}
	Σphenolic acids	45.36 ± 0.13	6.19 ± 0.03^{b}	7.57 ± 0.15^{a}	7.16 ± 0.04^{b}	10.93 ± 0.15^{a}	7.48 ± 0.02^{b}	18.99 ± 0.04^{a}	15.87 ± 0.01^{b}	30.09 ± 0.18^{a}	27.29 ± 0.01^{b}	46.49 ± 0.20^{a}
	Res	7.13 ± 0.09	1.46 ± 0.00^{b}	2.21 ± 0.02^{a}	1.55 ± 0.02^{b}	2.27 ± 0.02^{a}	2.12 ± 0.05^{b}	2.49 ± 0.01^{a}	3.17 ± 0.05^{a}	2.78 ± 0.04^{b}	7.77 ± 0.02^{a}	3.76 ± 0.04^{b}
70°C	Cat	46.02 ± 0.10	8.18 ± 0.01^{a}	6.09 ± 0.12^{b}	13.63 ± 0.04^{b}	15.62 ± 0.34^{a}	18.24 ± 0.01^{b}	21.98 ± 0.22^{a}	67.2 ± 0.02^{a}	33.02 ± 0.85^{b}	85.56 ± 0.02^{a}	55.77 ± 0.54^{b}
	Epi	78.25 ± 1.01	3.48 ± 0.05^{b}	6.69 ± 0.28^{a}	15.64 ± 0.01^{b}	22.42 ± 0.35^{a}	21.41 ± 0.04^{b}	32.04 ± 0.76^{a}	74.03 ± 0.02^{a}	49.67 ± 1.69^{b}	82.59 ± 0.02^{a}	87.24 ± 2.76^{a}
	Epig	29.50 ± 0.36	1.87 ± 0.00^{b}	2.42 ± 0.05^{a}	12.29 ± 0.03^{a}	6.58 ± 0.06^{b}	13.74 ± 0.04^{a}	10.17 ± 0.15^{b}	18.18 ± 0.06^{a}	13.62 ± 0.27^{b}	32.13 ± 0.02^{a}	23.04 ± 0.43^{b}
	EpiG	140.20 ± 0.04	27.82 ± 0.08^{a}	25.30 ± 0.23^{b}	97.36 ± 0.02^{a}	98.78 ± 0.27^{a}	141.41 ± 0.01^{a}	106.30 ± 0.31^{b}	456.26 ± 0.04^{a}	218.70 ± 0.09^{b}	556.97 ± 0.05^{a}	302.08 ± 2.23^{b}
	Σmonomerics	293.97 ± 0.38	41.35 ± 0.47^{a}	40.50 ± 0.17^{b}	138.92 ± 0.02^{b}	143.40 ± 0.25^{a}	194.80 ± 0.02^{a}	170.49 ± 0.36^{b}	615.67 ± 0.03^{a}	315.01 ± 0.72^{b}	757.25 ± 0.03^{a}	468.13 ± 1.49^{b}
	Pro B1	134.10 ± 1.15	6.41 ± 0.01^{b}	14.61 ± 0.40^{a}	25.43 ± 0.05^{b}	37.04 ± 0.88^{a}	50.54 ± 0.01^{a}	40.84 ± 0.41^{b}	173.11 ± 0.03^{a}	71.81 ± 1.19^{b}	279.59 ± 0.01^{a}	254.70 ± 3.97^{b}
	Pro B2	96.45 ± 1.05	12.31 ± 0.02^{b}	13.26 ± 0.18^{a}	24.59 ± 0.02^{b}	42.56 ± 0.98^{a}	34.18 ± 0.05^{b}	57.20 ± 1.56^{a}	99.14 ± 0.05^{a}	81.34 ± 0.28^{b}	144.21 ± 0.04^{a}	124.57 ± 3.18^{b}
	Σdimerics	230.55 ± 1.10	18.72 ± 0.01^{b}	27.87 ± 0.29^{a}	50.02 ± 0.03^{b}	79.60 ± 0.93^{a}	84.72 ± 0.03^{b}	98.04 ± 0.98^{a}	272.25 ± 0.04^{a}	153.15 ± 0.73^{b}	423.80 ± 0.02^{a}	379.27 ± 3.57^{b}
	G.A	22.42 ± 0.17	2.14 ± 0.04^{b}	2.48 ± 0.04^{a}	3.64 ± 0.05^{b}	6.41 ± 0.04^{a}	2.04 ± 0.00^{b}	9.41 ± 0.04^{a}	9.67 ± 0.01^{b}	14.49 ± 0.41^{a}	8.14 ± 0.01^{b}	34.98 ± 0.14^{a}
	F.A	20.15 ± 0.14	2.25 ± 0.00^{b}	3.29 ± 0.05^{a}	7.58 ± 0.02^{b}	15.61 ± 0.53^{a}	7.32 ± 0.02^{b}	20.05 ± 0.56^{a}	12.62 ± 0.05^{b}	23.28 ± 0.34^{a}	15.19 ± 0.01^{b}	27.22 ± 0.65^{a}
	C.A	2.79 ± 0.09	1.87 ± 0.00^{b}	2.63 ± 0.02^{a}	2.34 ± 0.05^{a}	2.44 ± 0.07^{a}	5.33 ± 0.01^{a}	4.73 ± 0.03^{b}	6.38 ± 0.01^{a}	6.06 ± 0.02^{b}	10.78 ± 0.05^{a}	9.66 ± 0.05^{b}
	Σphenolic acids	45.36 ± 0.40	6.26 ± 0.04^{b}	8.40 ± 0.04^{a}	13.56 ± 0.04^{b}	24.46 ± 0.21^{a}	14.69 ± 0.01^{b}	34.19 ± 0.21^{a}	28.67 ± 0.02^{b}	43.83 ± 0.26^{a}	34.11 ± 0.02^{b}	71.86 ± 0.28^{a}
	Res	7.13 ± 0.09	1.46 ± 0.00^{b}	1.95 ± 0.02^{a}	2.10 ± 0.05^{b}	2.53 ± 0.00^{a}	2.45 ± 0.03^{b}	3.22 ± 0.11^{a}	5.86 ± 0.01^{a}	3.11 ± 0.00^{b}	15.35 ± 0.01^{a}	5.82 ± 0.05^{b}

Mean (n =3) ± SD. For each maceration time from the two vintages, different letters in the same row indicate significant difference at $p < 0.05$. Cat, catechin; Epi, epicatechin; Epig, epicatechin gallte; EpiG, epigallocatechin; Pro B1, procyanidin B1; Pro B2, procyanidin B2; G.A., gallic acid; F.A., ferulic acid; C.A., caffeic acid; Res, resveratrol; CT-014, Cabernet Sauvignon Saint Thomas 2014; n.d., not detected values

II.2.3.2. IMPACT OF MACERATING ENZYMES ON POLYPHENOL COMPOSITION OF MUSTS FROM 2015 VINTAGE

The kinetics of extraction and evolution of chromatic parameters and phenolic composition during the enzymatic macerations at 70°C and 25°C of Syrah and Cabernet Sauvignon varieties were respectively shown in tables II.2.5-a and II.2.5-b. The results demonstrated that the addition of a pectolytic enzyme to the maceration musts affects their contents in tannins and anthocyanins and accelerates their extraction. For Syrah, the total anthocyanins reached their maximum after 8 hours of maceration with concentrations of 485.33 mg/l and 417.37 mg/l respectively for the must treated and untreated with enzymes. Similarly, the maximum tannin concentration reached after 24 hours was higher in enzyme-added musts (9426.59 mg/l with enzyme and 6746.17 mg/l without enzyme). Similar results were observed for the Cabernet Sauvignon musts where the extraction of polyphenols is favored by the addition of enzymes. Subsequently, the must treated with maceration enzymes showed maximum concentrations of 498.17 mg/l of total anthocyanins and 8228.14 mg/l of total tannins compared to untreated musts with respective values of 417.37 mg/l and 7377.62 mg/l. Contrary to these results, other studies conducted by parley et al. (2001) and Wightman et al. (1997) showed that pectinase enzyme addition did not increase the anthocyanin extraction but did increase the formation of polymeric pigments. Degradation of total anthocyanins is noticed after 8 hours of maceration due to the effect of heat. This decrease reached 27.16% for Cabernet Sauvignon and 30.58% for Syrah. In addition, color intensity, increases progressively during the 8 hours (showed the same trend than anthocyanins) and reaches higher values for the musts treated with the maceration enzyme (1.99 for Syrah and 2.07 for Cabernet Sauvignon). The qualitative analysis of the total polyphenols showed a similar effect of the pectolytic enzyme. Subsequently, maximum values of TPI with added enzyme were on average 1.67 times higher for the two grape musts compared to those without added enzymes at the same maceration time. Similar results are found for total polyphenols. Maximum concentrations, expressed in mg/l GAE were 4195.00 and 4820.00 mg/l respectively for Cabernet Sauvignon and Syrah must with added enzymes after 24h. For the macerated and fermented juices from the two varieties (control), the same observation is observed for the effect of the maceration enzyme. Its addition improves significantly total anthocyanins, total tannins, color intensity, total polyphenol index and total polyphenol concentrations compared to control without added enzymes. CS control values with added enzymes were +13.57%; +13.16%;

+15.19% and +25.25% higher respectively for TA; TPI; TP and tannins than for CS control without enzyme. Values of Sy control with maceration enzymes were +7.1%; +18.12%; +9.63%; +5.26% and +30.64% higher respectively for TA; CI; TPI; TP and T than Sy control without enzymes. Syrah and CS macerated at 70°C with added enzymes after 24 hours showed higher values than their respective controls with added enzymes, average values for the two grape musts were respectively 1.52; 1.32; 1.58; 1.18 and 4.15 times higher respectively for TA; CI; TPI; TP and T.

Concerning HPLC phenolic compounds, the results of Tables II.2.5-a and II.2.5-b showed that the extraction of individual anthocyanin compounds like total anthocyanins is favored by the maceration enzymes addition. Malvidin-3-O-glucoside, being the most represented compound among anthocyanins, reaches its maximum values of 133.26 mg/l (+ 66.53% more than 70°C without added enzyme) and 101.42 mg/l (+ 15.58% more than 70°C without added enzyme) after 8 hours of maceration respectively for Syrah and Cabernet Sauvignon. The evolution of cyanidin-3-O-glucoside in Cabernet Sauvignon remains very low over time, whereas it reaches significant maximums values of 4.88 mg/l and 4.76 mg/l in Syrah musts. The presence of peonidin-3-O-glucoside is much more important in Syrah (24.97 mg/l) than in Cabernet Sauvignon (6.93 mg/l) while that of delphinidin-3-O-glucoside were nearly the same. The prolongation of the maceration causes degradation of the anthocyanidic compounds under the effect of heat reaching 45% on certain compounds for Cabernet Sauvignon and 58% for Syrah. In addition, Syrah and Cabernet Sauvignon musts added with maceration enzymes and fermented by Y strain revealed higher individual anthocyanins than those fermented without added enzymes ($[Dp]_{Sy\text{-}E}$ = 9.60 mg/l ; $[Cy]_{Sy\text{-}E}$ = 3.43 mg/l; $[Pn]_{Sy\text{-}E}$ = 7.99 mg/l; $[Mv]_{Sy\text{-}E}$ = 71.26 mg/l; $[Dp]_{CS\text{-}E}$ = 6.23 mg/l; $[Cy]_{CS\text{-}E}$ = 2.36 mg/l; $[Pn]_{CS\text{-}E}$ = 3.63 mg/l; $[Mv]_{CS\text{-}E}$ = 75.73 mg/l). Sy and CS control with enzymes showed average values of 3.49; 1.44 and 1.33 times higher respectively for Dp, Cy and Mv than those of their respective Sy and CS musts macerated at 70°C with enzymes after 24 hours.

As for monomeric and dimeric tannins from the two grape varieties, their extraction is favored by the addition of pectolytic enzymes. Epigallocatechin is the most represented monomer with maximum concentrations of 295.67 mg/l and 310.58 mg/l and respectively for Syrah and Cabernet Sauvignon musts. For the other monomers, the addition of maceration enzymes to the must increases yields of 3.83%; 10.87% and 66.14% respectively for catechin, epicatechin and

Table II.2.5-a: Chromatic parameters and phenolic composition of Syrah musts and Syrah control (25°C) from the 2014 vintage with and without added enzymes in terms of time and temperature

	Sy-maceration time (hours)											
			0		2		4		8		24	
	Control 25°C	Control 25°C + enzyme	70°C	70°C + enzyme	70°C	70°C + enzyme	70°C	70°C + enzyme	70°C	70°C + enzyme	70°C	70°C + enzyme
TA	220.25 ± 13.47^{b}	244.71 ± 4.40^{a}	26.54 ± 3.07^{b}	82.25 ± 2.32^{a}	101.21 ± 3.07^{b}	258.71 ± 0.51^{a}	389.37 ± 0.88^{b}	468.12 ± 3.50^{a}	417.37 ± 0.87^{b}	485.33 ± 1.82^{a}	337.75 ± 3.03^{a}	336.87 ± 3.83^{a}
CI	1.22 ± 0.01^{b}	1.49 ± 0.03^{a}	0.16 ± 0.02^{b}	0.76 ± 0.02^{a}	0.66 ± 0.02^{b}	1.93 ± 0.02^{a}	1.16 ± 0.06^{b}	2.12 ± 0.03^{a}	1.51 ± 0.09^{b}	1.99 ± 0.04^{a}	1.46 ± 0.09^{b}	1.82 ± 0.02^{a}
TPI	60.12 ± 2.57^{b}	66.53 ± 3.08^{a}	12.33 ± 0.21^{b}	17.53 ± 0.40^{a}	32.37 ± 0.46^{b}	56.33 ± 0.40^{a}	40.40 ± 0.26^{b}	72.03 ± 0.49^{a}	45.30 ± 0.62^{b}	78.80 ± 0.62^{a}	60.47 ± 1.97^{b}	99.40 ± 0.61^{a}
TP	2452.25 ± 46.19^{b}	2588.33 ± 23.12^{a}	402.67 ± 12.58^{b}	973.33 ± 41.93^{a}	1475.00 ± 13.23^{b}	2608.33 ± 41.93^{a}	2051.67 ± 2.88^{b}	3431.67 ± 12.58^{a}	2576.67 ± 5.77^{b}	4021.67 ± 10.40^{a}	3468.33 ± 2.88^{b}	4820.00 ± 165.22^{a}
T	1154.68 ± 62.14^{b}	1664.95 ± 21.24^{a}	940.77 ± 29.53^{b}	1701.04 ± 19.33^{a}	2358.26 ± 88.58^{b}	4574.55 ± 19.33^{a}	3311.87 ± 93.35^{b}	5940.75 ± 230.613^{a}	3595.38 ± 33.48^{b}	8054.17 ± 146.36^{a}	6746.17 ± 165.15^{b}	9426.59 ± 327.09^{a}
Dp	6.00 ± 0.18^{a}	9.60 ± 0.26^{a}	1.62 ± 0.03^{a}	1.70 ± 0.04^{a}	1.82 ± 0.05^{a}	1.79 ± 0.01^{a}	1.87 ± 0.04^{a}	1.92 ± 0.06^{a}	2.33 ± 0.06^{a}	2.18 ± 0.00^{b}	2.14 ± 0.01^{a}	2.14 ± 0.03^{a}
Cy	3.12 ± 0.04^{a}	3.43 ± 0.14^{a}	1.32 ± 0.04^{b}	1.90 ± 0.02^{a}	2.65 ± 0.00^{a}	2.49 ± 0.03^{b}	3.19 ± 0.07^{b}	4.34 ± 0.03^{a}	4.88 ± 0.05^{a}	4.76 ± 0.01^{b}	2.71 ± 0.05^{a}	2.65 ± 0.02^{a}
Pn	6.10 ± 0.13^{a}	7.99 ± 0.11^{a}	3.12 ± 0.01^{b}	6.71 ± 0.19^{a}	11.01 ± 0.22^{b}	14.57 ± 0.11^{a}	13.55 ± 0.32^{b}	22.37 ± 0.34^{a}	16.36 ± 0.35^{a}	24.97 ± 0.50^{b}	12.51 ± 0.25^{a}	10.55 ± 0.23^{b}
Mv	65.35 ± 0.51^{a}	71.26 ± 0.51^{a}	7.87 ± 0.04^{b}	34.62 ± 0.21^{a}	55.41 ± 0.66^{b}	76.92 ± 0.99^{a}	88.24 ± 1.35^{b}	122.57 ± 1.98^{a}	44.6 ± 1.60^{b}	133.26 ± 1.65^{a}	10.65 ± 2.05^{b}	54.96 ± 0.78^{a}
Cat	53.00 ± 0.34^{b}	62.65 ± 0.13^{a}	19.73 ± 0.53^{a}	10.23 ± 0.34^{b}	33.03 ± 0.92^{a}	25.00 ± 1.35^{b}	16.55 ± 2.31^{b}	52.61 ± 0.83^{a}	43.86 ± 0.72^{b}	75.12 ± 1.91^{a}	108.72 ± 0.64^{b}	113.05 ± 0.76^{a}
Epi	90.22 ± 0.76^{b}	98.83 ± 0.31^{a}	17.80 ± 1.00^{a}	13.88 ± 0.43^{b}	29.48 ± 1.05^{a}	30.42 ± 0.37^{a}	43.66 ± 2.91^{b}	70.38 ± 0.53^{a}	56.54 ± 1.04^{b}	91.64 ± 0.66^{a}	135.33 ± 2.72^{b}	151.83 ± 1.90^{a}
Epig	22.13 ± 0.89^{a}	19.19 ± 0.54^{b}	5.87 ± 0.33^{a}	4.18 ± 0.15^{b}	6.38 ± 0.59^{b}	11.64 ± 0.30^{a}	9.69 ± 0.12^{b}	10.59 ± 0.20^{a}	13.94 ± 0.79^{b}	25.64 ± 0.74^{a}	18.36 ± 2.22^{b}	54.22 ± 0.12^{a}
EpiG	72.32 ± 0.29^{b}	80.42 ± 0.72^{a}	41.39 ± 0.66^{a}	27.00 ± 1.32^{b}	33.87 ± 2.50^{a}	33.34 ± 1.55^{a}	54.63 ± 0.32^{b}	59.90 ± 1.21^{a}	129.34 ± 0.85^{b}	223.94 ± 1.34^{a}	347.74 ± 2.47^{a}	295.67 ± 1.06^{b}
Pro B1	110.05 ± 0.28^{b}	114.78 ± 0.84^{a}	9.33 ± 0.51^{b}	18.95 ± 0.37^{a}	21.73 ± 1.89^{b}	35.29 ± 0.51^{a}	30.68 ± 0.37^{b}	56.17 ± 1.71^{a}	101.53 ± 1.23^{b}	184.86 ± 2.46^{a}	161.05 ± 1.14^{b}	244.91 ± 1.64^{a}
Pro B2	115.32 ± 0.32^{b}	124.23 ± 0.61^{a}	24.85 ± 1.14^{a}	10.11 ± 0.49^{b}	41.16 ± 1.60^{a}	16.43 ± 0.85^{b}	72.58 ± 2.03^{a}	73.91 ± 3.14^{a}	103.61 ± 0.75^{b}	137.98 ± 0.01^{a}	134.21 ± 0.64^{b}	184.41 ± 0.98^{a}
GA	25.10 ± 0.10^{b}	26.88 ± 0.52^{a}	5.71 ± 0.50^{a}	4.42 ± 0.07^{b}	5.52 ± 0.66^{b}	7.33 ± 0.23^{a}	6.43 ± 0.18^{b}	9.37 ± 0.08^{a}	6.01 ± 0.44^{b}	11.74 ± 0.83^{a}	29.45 ± 0.22^{b}	33.99 ± 0.83^{a}
FA	60.22 ± 0.40^{b}	154.93 ± 0.72^{a}	7.27 ± 0.02^{b}	11.56 ± 0.33^{a}	24.81 ± 0.43^{a}	21.34 ± 0.77^{b}	26.31 ± 0.61^{b}	65.58 ± 0.94^{a}	61.95 ± 1.41^{b}	72.07 ± 0.93^{a}	80.20 ± 2.75^{a}	67.23 ± 1.56^{b}
CA	25.08 ± 0.15^{a}	3.20 ± 0.10^{b}	3.56 ± 0.31^{a}	3.24 ± 0.07^{a}	3.69 ± 0.08^{b}	7.05 ± 0.04^{a}	4.19 ± 0.00^{b}	12.92 ± 0.67^{a}	6.32 ± 0.09^{b}	14.74 ± 0.51^{a}	9.45 ± 0.20^{b}	22.73 ± 0.15^{a}
Res	7.14 ± 0.00^{b}	15.70 ± 0.57^{a}	2.51 ± 0.13^{a}	2.52 ± 1.52^{a}	2.80 ± 0.08^{b}	3.39 ± 1.11^{a}	4.48 ± 0.28^{a}	4.33 ± 0.13^{a}	4.12 ± 0.20^{a}	3.55 ± 0.21^{b}	6.84 ± 0.03^{b}	9.10 ± 0.00^{a}

Mean (n =3) ± SD. For each maceration time, different letters in the same row indicate significant difference at $p < 0.05$. TA, total anthocyanins,; CI, Color intensity; TPI, total phenolic index; TP, total phenolic; T, tannins; Dp, delphinidin-3-O-glucoside; Cy, cyanidin-3-O-glucoside; Pn, peonidin-3-O-glucoside; Mv, malvidin-3-O-glucoside, Cat, catechin; Epi, epicatechin; Epig, epicatechin gallte; EpiG, epigallocatechin; Pro B1, procyanidin B1; Pro B2, procyanidin B2; GA., gallic acid; FA., ferulic acid; CA., caffeic acid; Res, resveratrol

Table II.2.5-b: Chromatic parameters and phenolic composition of Cabernet Sauvignon musts and Cabernet Sauvignon control (25°C) from the 2014 vintage with and without added enzymes in terms of time and temperature

	CS-maceration time (hours)											
			0		2		4		8		24	
	Control 25°C	Control 25°C + enzyme	70°C	70°C + enzyme	70°C	70°C + enzyme	70°C	70°C + enzyme	70°C	70°C + enzyme	70°C	70°C + enzyme
TA	187.54 ± 0.50^{b}	217.00 ± 2.31^{a}	4.37 ± 0.00^{b}	28.00 ± 0.00^{a}	227.21 ± 7.44^{b}	244.42 ± 3.07^{a}	253.17 ±7.44^{b}	483.88 ± 8.88^{a}	374.79 ± 9.19^{b}	498.17 ± 0.50^{a}	292.25 ± 0.87^{b}	362.83 ± 1.34^{a}
CI	1.20 ± 0.01^{a}	1.18 ± 0.05^{a}	0.13 ± 0.00^{b}	1.05 ± 0.02^{a}	0.57 ± 0.04^{b}	1.95 ± 0.01^{a}	0.87 ± 0.01^{b}	2.04 ± 0.01^{a}	1.17 ± 0.02^{b}	2.07 ± 0.03^{a}	1.26 ± 0.01^{b}	1.69 ± 0.00^{a}
TPI	50.19 ± 0.04^{b}	57.80 ± 0.10^{a}	11.47 ± 0.30^{b}	22.37 ± 0.30^{a}	24.97 ± 0.76^{b}	59.30 ± 0.36^{a}	28.70 ± 0.35^{b}	70.73 ± 0.55^{a}	38.10 ± 0.37^{b}	78.23 ± 0.60^{a}	56.60 ± 2.07^{b}	96.97 ± 2.28^{a}
TP	2250.35 ± 5.77^{b}	2653.33 ± 5.77^{a}	763.33 ± 2.33^{a}	688.33 ± 0.20^{b}	1350.00 ± 1.41^{b}	1870.00 ± 1.33^{a}	1806.67 ± 0.92^{b}	2511.67 ± 1.58^{a}	1883.33 ± 1.77^{b}	2928.33 ± 2.63^{a}	3180.00 ± 1.02^{b}	4195.00 ± 5.14^{a}
T	2330.99 ± 1.71^{b}	3118.57 ± 77.66^{a}	2081.20 ± 2.64^{a}	2055.42 ± 3.18^{a}	3634.04 ± 5.31^{b}	3981.98 ± 0.37^{a}	3788.68 ± 7.57^{b}	4838.94 ± 5.76^{a}	5167.55 ± 2.49^{b}	7036.12 ± 0.00^{a}	7377.62 ± 3.37^{b}	8228.14 ± 3.18^{a}
Dp	4.63 ± 0.30^{b}	6.23 ± 0.02^{a}	n.d	1.68 ± 0.00^{a}	n.d	1.63 ± 0.03^{a}	1.63 ± 0.01^{b}	1.78 ± 0.05^{a}	1.88 ± 0.00^{b}	2.23 ± 0.00^{a}	2.27 ± 0.01^{b}	2.46 ± 0.04^{a}
Cy	1.91 ± 0.00^{b}	2.36 ± 0.08^{a}	1.26 ± 0.04^{b}	1.35 ± 0.00^{a}	1.60 ± 0.06^{a}	1.39 ± 0.01^{b}	1.65 ± 0.06^{a}	1.44 ± 0.01^{b}	1.64 ± 0.04^{b}	1.75 ± 0.02^{a}	1.41 ± 0.04^{b}	1.48 ± 0.02^{a}
Pn	2.92 ± 0.01^{b}	3.63 ± 0.13^{a}	1.14 ± 0.01^{b}	1.85 ± 0.03^{a}	4.84 ± 0.11^{a}	2.37 ± 0.06^{b}	5.54 ± 0.01^{a}	4.74 ± 0.03^{b}	6.79 ± 0.17^{a}	6.93 ± 0.01^{a}	4.87 ± 0.04^{b}	5.62 ± 0.11^{a}
Mv	66.35 ± 1.98^{b}	75.73 ± 1.02^{a}	10.99 ± 0.57^{b}	20.57 ± 0.53^{a}	60.79 ± 0.92^{a}	29.21 ± 0.81^{b}	77.14 ± 1.88^{a}	66.53 ± 0.63^{b}	85.62 ± 2.19^{b}	101.42 ± 1.48^{a}	54.54 ± 1.41^{a}	55.16 ± 0.28^{a}
Cat	46.02 ± 0.10^{b}	68.12 ± 1.24^{a}	6.09 ± 0.12^{b}	6.64 ± 0.18^{a}	15.62 ± 0.34^{a}	7.93 ± 0.07^{b}	21.98 ± 0.22^{b}	42.82 ± 0.13^{a}	33.02 ± 0.85^{b}	62.47 ± 0.55^{a}	55.77 ± 0.54^{b}	83.84 ± 0.92^{a}
Epi	78.25 ± 1.01^{b}	89.28 ± 1.00^{a}	6.69 ± 0.28^{b}	7.34 ± 0.05^{a}	22.42 ± 0.35^{a}	22.38 ± 0.14^{a}	32.04 ± 0.76^{b}	66.27 ± 0.36^{a}	49.67 ± 1.69^{b}	92.37 ± 1.84^{a}	87.24 ± 2.76^{b}	120.79 ± 0.84^{a}
Epig	29.50 ± 0.36^{a}	7.02 ± 0.02^{b}	2.42 ± 0.05^{b}	2.58 ± 0.06^{a}	6.58 ± 0.06^{a}	3.51 ± 0.13^{b}	10.17 ± 0.15^{a}	5.20 ± 0.16^{b}	13.62 ± 0.27^{b}	19.47 ± 0.02^{a}	23.04 ± 0.43^{b}	30.64 ± 0.33^{a}
EpiG	140.20 ± 0.04^{a}	65.64 ± 0.08^{b}	25.30 ± 0.23^{b}	28.00 ± 1.33^{a}	98.78 ± 0.27^{a}	43.00 ± 0.77^{b}	106.30 ± 0.31^{a}	69.62 ± 2.47^{b}	218.70 ± 0.09^{b}	310.58 ± 0.50^{a}	302.08 ± 2.23^{a}	289.29 ± 4.91^{b}
Pro B1	134.10 ± 1.15^{a}	132.60 ± 1.63^{b}	14.61 ± 0.40^{b}	18.52 ± 0.41^{a}	37.04 ± 0.88^{b}	41.96 ± 0.02^{a}	40.84 ± 0.41^{b}	67.99 ± 0.41^{a}	71.81 ± 1.19^{b}	148.47 ± 0.33^{a}	254.70 ± 3.97^{b}	339.22 ± 0.65^{a}
Pro B2	96.45 ± 1.05^{b}	110.64 ± 0.45^{a}	13.26 ± 0.18^{b}	17.90 ± 0.03^{a}	42.56 ± 0.98^{a}	23.35 ± 0.55^{b}	57.20 ± 1.56^{a}	49.18 ± 0.18^{b}	81.34 ± 0.28^{b}	104.24 ± 1.17^{a}	124.57 ± 3.18^{b}	137.85 ± 1.19^{a}
GA	22.42 ± 0.17b	25.83 ± 0.45^{a}	2.48 ± 0.04^{b}	3.40 ± 0.11^{a}	6.41 ± 0.04^{a}	3.94 ± 0.02^{b}	9.41 ± 0.04^{a}	5.36 ± 0.22^{b}	14.49 ± 0.41^{b}	29.76 ± 0.15^{a}	34.98 ± 0.14^{b}	40.81 ± 0.13^{a}
FA	20.15 ± 0.14^{b}	71.60 ± 1.00^{a}	3.29 ± 0.05^{b}	4.12 ± 0.03^{a}	15.61 ± 0.53^{b}	18.25 ± 0.01^{a}	20.05 ± 0.56^{b}	22.84 ± 1.01^{a}	23.28 ± 0.34^{b}	25.73 ± 0.34^{a}	27.22 ± 0.65^{a}	24.98 ± 0.61^{b}
CA	2.79 ± 0.09^{a}	2.81 ± 0.26^{a}	2.63 ± 0.02^{b}	4.32 ± 0.06^{a}	2.44 ± 0.07^{b}	5.23 ± 0.06^{a}	4.73 ± 0.03^{b}	12.42 ± 0.04^{a}	6.06 ± 0.02^{b}	13.06 ± 0.03^{a}	9.66 ± 0.05^{b}	20.42 ± 0.06^{a}
Res	7.13 ± 0.09^{b}	11.13 ± 0.88^{a}	1.95 ± 0.02^{b}	2.17 ± 0.02^{a}	2.53 ± 0.00^{a}	1.98 ± 0.01^{b}	3.22 ± 0.11^{a}	2.77 ± 0.03^{b}	3.11 ± 0.00^{a}	2.86 ± 0.03^{b}	5.82 ± 0.05^{b}	6.95 ± 0.01^{a}

Mean (n =3) ± SD. For each maceration time, different letters in the same row indicate significant difference at $p < 0.05$. TA, total anthocyanins,; CI, Color intensity; TPI, total phenolic index; TP, total phenolic; T, tannins; Dp, delphinidin-3-O-glucoside; Cy, cyanidin-3-O-glucoside; Pn, peonidin-3-O-glucoside; Mv, malvidin-3-O-glucoside, Cat, catechin; Epi, epicatechin; Epig, epicatechin gallte; EpiG, epigallocatechin; Pro B1, procyanidin B1; Pro B2, procyanidin B2; GA., gallic acid; FA., ferulic acid; CA., caffeic acid; Res, resveratrol

epicatechin gallate in Syrah, as well as, 33.48%; 27.77% and 24.80% respectively in Cabernet Sauvignon after 24 hours of maceration. Among the dimeric tannins, procyanidin B1 is the most represented. Its maximum values were increased by 24.91% and 34.24% by the addition of maceration enzymes to the Cabernet Sauvignon and Syrah musts respectively. Similar results for procyanidin B2, with percentage increase of 9.63% and 27.22% respectively. As for the extraction of tannins, the addition of the pectolytic enzyme increases the levels of phenolic acids obtained after 24 hours of maceration. Thus, gallic acid and caffeic acid have respective maximum value of 40.81 mg/l (+14.28%) and 20.42 mg/l (+52.69%) for Cabernet Sauvignon, and 33.99 mg/l (+13.36%) and 22.73 mg/l (58.42%) for Syrah in the presence of enzymes. Ferulic acid shows contradictory results in both grape varieties. Same as for phenolic acids, the highest level of resveratrol was obtained with the use of maceration enzymes (+24.83% for Syrah (9.10 mg/l) and +6.25% for Cabernet Sauvignon (6.95 mg/l)). Similarly results were observed for Sy and CS controls with maceration enzymes. Epigallocatechin and procyanidin B1 remains the most represented monomers and dimers in both grape varieties. For Syrah, an increase of 15.40% was observed for the Cat, 8.71% for Epi; 10.07% for the EpiG, 4.12% for the Pro B1 as well as 7.17% for the Pro B2. With few exceptions, the same results were observed for Cabernet Sauvignon control with added enzymes with different percentages. Moreover, the extraction of phenolic acids and resveratrol was also improved by the use of maceration enzymes. Ferulic acid is the most represented with an increase value of 61.13% and 71.85% for Sy and CS respectively. Excepting for resveratrol Sy and CS macerated at 70°C + enzymes showed higher values than those of their respective controls with added enzymes. After all, as seen from our results (Tables II.2.5-a and II.2.5-b), maceration enzymes addition (70°C and 25°C + enzyme), promoted higher concentration of TA, CI, TPI, TP, T and HPLC phenolic profiles than macerating at the same temperature without added enzymes. In fact, the higher value of phenolic compounds of enzyme-treated musts was achieved because macerating enzymes, by degrading the cell walls, favor tissue degradation and the dissolution of the cell wall contents, including anthocyanins and other phenolic compounds, especially tannins. These results are in accordance with those observed by Parley (1997) and Padro et al. (1999). They tested several enzyme preparations and all of them produced an increase in the quantity of polyphenols extracted from the solid parts.

II.2.3.3. IMPACT OF MACERATION TIME AND TEMPERATURE ON BIOLOGICAL ACTIVITIES

By comparing the different biological activities found in the two grape must varieties at two consecutives vintages after 48 and 24h of maceration respectively for 2014 and 2015 vintage at different temperatures (60°C, 70°C and 25°C), Figure II.2.2-a showed that Syrah macerated at 60°C for the two consecutive vintages had the same antioxidant activities (ABTS and DPPH), in addition to the presence of low percent inhibition rates for anti-LOX (11.30%) and HCT116 (15.10%) activity for Syrah 2014. This can be due as seen in Figure II.2.3-a to their highest content of resveratrol. In fact studies conducted by Baur et al. (2006) and Kris-Etherton et al., (2002) and Tredici et al. (1999) have shown that resveratrol possess diverse biological activities that confer protection against oxidative stress, inflammmation, aggregate functions, cardiovascular disease, neurodegenerative disorders and cancer (such as skin cancers and tumors of the gastrointestinal tract). Besides, Sy-70°C-2014 showed percentage of inhibition 2.11; 3.1; 20.14 and 47.60 times higher respectively for ABTS, DPPH, LOX and HCT116 than for Sy-70°C-2015, whereas, anti α-glucosidase and anti ChE activity percentage inhibition value was almost the same for the two vintages. Furthermore, Syrah control showed 1.46 times higher antidiabetic activities than Sy-ST macerated at 70°C for the two vintages which may be the result of it is high anthocyanin and gallic acid content (Figure II.2.3-a). These compounds according to the other studies (Sri Balasubashini et al., 2003 and Zunino, 2009) have been shown to inhibit hyperglycemia. As to CS vintages, Figure II.2.2-b showed that CS-60°C-2015 presented slightly higher values of ABTS and DPPH than CS-60°C-2014, while this latter presented percentage inhibition value of approximately 7% respectively for LOX and ChE activity and 9.6% for HCT116 activity which can be due as seen previously to their highest content of resveratrol. In other hand, CS-70°C-2014 presented higher values of ABTS, DPPH, LOX and same values of anti-α-glucosidase than CS-70°C-2015. Values were 1.29 and 1.12 times higher respectively for ABTS and DPPH and 2.07 times higher for LOX. Low percent inhibition of ChE (5.98%) and HCT116 (3.30%) were present in CS of the 2014 vintage. Finally, CS control showed 2.41 and 5.51 times higher anti LOX and anti-α-glucosidase activity than CS-70°C-2014, which can be due to their higher content of anthocyanins and gallic acid (Figure II.2.3-b). These compounds as seen previously (II.1.3.2. p. 111) have been shown to inhibit hyperglycemia. After all, as seen in Figure II.2.2-a and II.2.2-b must grapes macerated at 70°C for 48 hours presented higher

percentage and different types of biological activities for whatever the grape variety and the vintage.

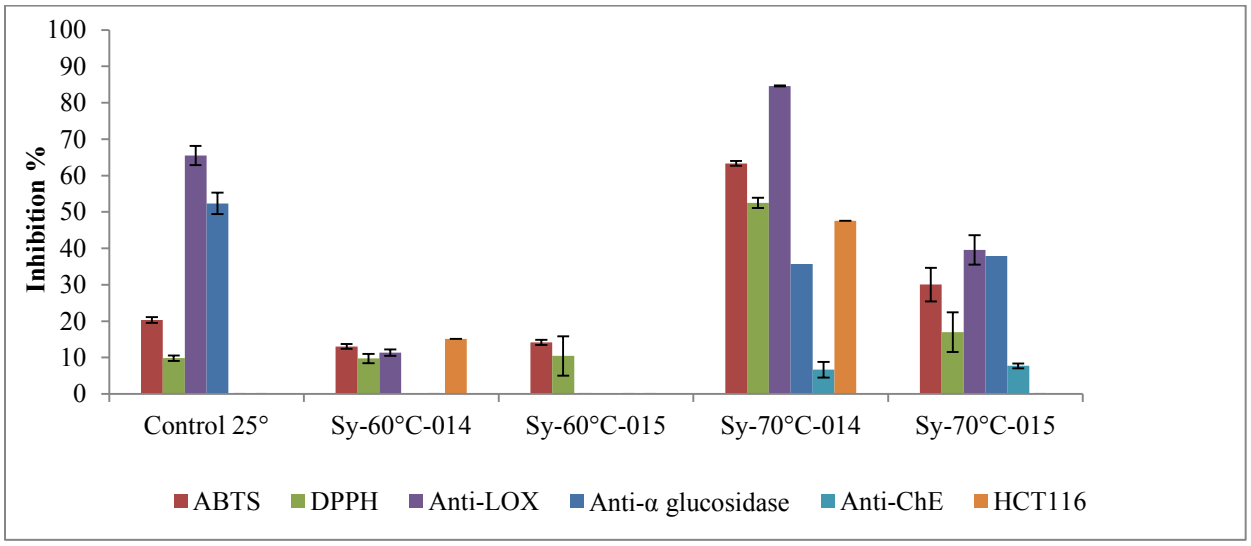

Figure II.2.2-a: Biological activities (ABTS and DPPH (antioxidant), Anti-LOX (antiinflammatory), Anti-α glucosidase (antidiabetic), Anti-ChE (antialzheimer) and HCT116 (anticancer)) of Sy-014 (Syrah 2014 vintage) and Sy-015 (Syrah 2015 vintage) grape musts macerated at different temperatures (60°C and 70°C) after 48 and 24 hours respectively for Syrah 2014 and 2015 vintage and for the control (Sy-015-25°C) after alcoholic fermentation. Data were expressed as mean percentage of inhibition (inhibition %) ± standard deviation.

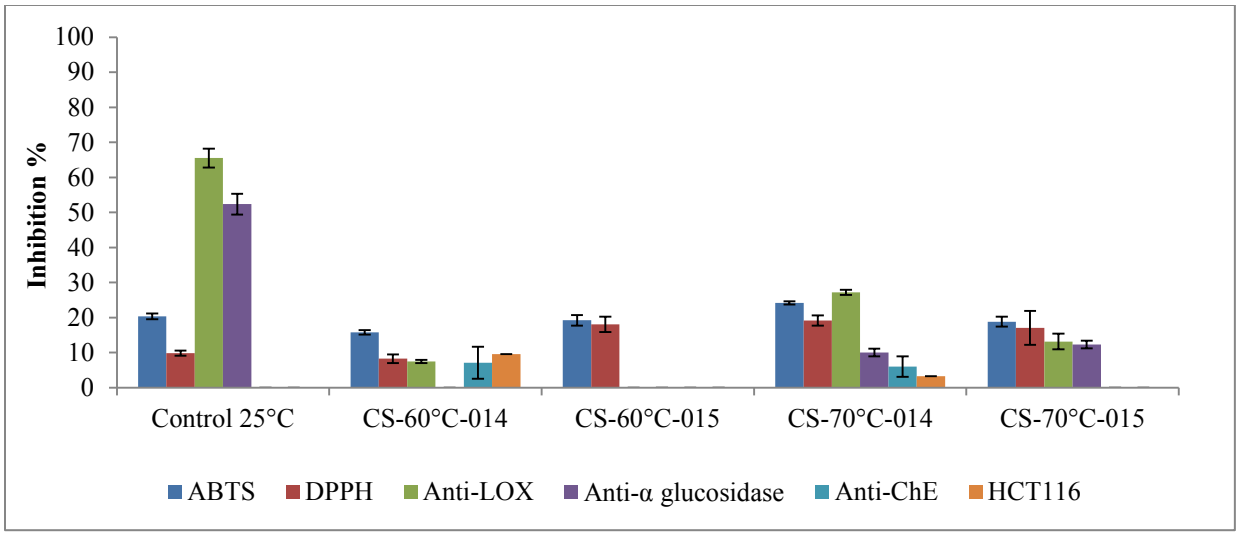

Figure II.2.2-b: Biological activities (ABTS and DPPH (antioxidant), Anti-LOX (antiinflammatory), Anti-α glucosidase (antidiabetic), Anti-ChE (antialzheimer) and HCT116 (anticancer)) of CS-014 (Cabernet Sauvignon 2014 vintage) and CS-015 (Cabernet Sauvignon 2015 vintage) grape musts macerated at different temperatures (60°C and 70°C) after 48 and 24 hours respectively for Cabernet Sauvignon 2014 and 2015 vintage and for the control (CS-015-25°C). Data were expressed as mean percentage of inhibition (inhibition %) ± standard deviation

II.2.4. Vintage effect on phenolic composition of Syrah and Cabernet Sauvignon musts: comparison between 2014 and 2015 vintage and correlation with climatic indexes

In order to illustrate vintage and ripening effect of the two grape varieties from the two consecutive vintages, principal component analysis was performed. Figure II.2.3-a, showed the PCA biplot for the first two principal component analysis which explain 77.79% of the total variance. The first component is positively represented by the variables TA, CI, TPI, TP, T, Dp, Pro B1, EpiG, Cat, ProB2, C.A, Epi, Epig and Res. The second component is positively represented by F.A, Cy, Pn, Mv and G.A. Figure 3-b showed the PCA biplot for the first two principal component analysis which explain 80.01% of the total variance. The first component is positively represented by the variables TA, CI, TPI, TP, T, Dp, Cy, Pro B1, EpiG, Cat, ProB2, C.A, Epi, Epig, F.A and Res. The second component is positively represented by Pn Mv and GA. The projection of the 2014 and 2015 vintage of Syrah and Cabernet Sauvignon must samples over maceration time (0, 2, 4, 8, 24 and 48h) at different temperatures (60°C, 70°C and 25°C), showed similar evolution over time for the two vintages with different concentrations in

phenolics compounds. Vintage effect was observed on each studied phenolic compound concentration and was more important for Syrah than for Cabernet Sauvignon. So as to understand this effect, we look toward meteorological data (temperature and precipitation, LARI weather station). Averaged temperatures from May to September were set at 22.4°C for the two vintages. Moreover, in 2014, cumulated precipitation 60 days before flowering were set at 137.2 mm with total annual precipitation of 366.2 mm, whereas they were from 72.2 mm in 2015 with total annual precipitation of 228.6 mm. Since the average temperature was the same for the two vintages, the limiting factor will be vine water deficit. According to several studies (Ojeda et al. 2002; Roby et al. 2004), vine water deficit was first considered because of it is related impact on phenolic biosynthesis depending on water deficit period (flowering, veraison, harvest stage). In CS vine, the flavonoid pathway responsible for tannin and anthocyanin synthesis was shown to occur really early, as soon as the flowering stage and at the beginning of berry growth (Gagné et al. 2009). The water deficit observed at the flowering stage could be correlated to an increase of ABA levels, a key regulator of berry ripening, strongly involved in the control of the proanthocyanidin pathway and would have a positive impact on tannin and anthocyanin biosynthesis from the flowering stage (Koyoma et al. 2010; Lacampagne et al., 2010) and consistent with an activation of the flavonoid pathway leading to more important phenol concentrations. Moreover, higher levels of TA, CI, TPI, and flavanols were observed in wine made from the mature grapes (Gómez-Plaza et al., 2001; Gil et al. 2012). Thus low concentrations in phenolic compounds for 2015 vintage could find another explanation in this last comment. The water deficit and the highest degree of ripening of the 2015 vintage comparing to 2014 was not correlated with the higher concentrations of phenolic compounds. Then to understand this effect we turned towards some particular weather conditions, an unseasonal sandstorm hits the Bekaa valley in eastern Lebanon. These climatic conditions could have induced damage in anthocyanins and tannins, reducing their amounts. Other studies conducted by Chorti et al. 2010 indicated that sunlight exposure (other climatic conditions), essential for grape berry ripening could also be responsible for excessive sunburn and qualitative and quantitative vine damages especially on anthocyanins accumulation of Nebbiolo grapes skins. The effect of sandstorm was more damaging in Syrah than for Cabernet Sauvignon. This may be due both to the delayed maturation and thickness of grapes skins between the two

varieties (CS had higher ratio of solids (skins plus seeds) to liquid (pulp or juice) (Pérez-Magariño and González-San José, 2004).

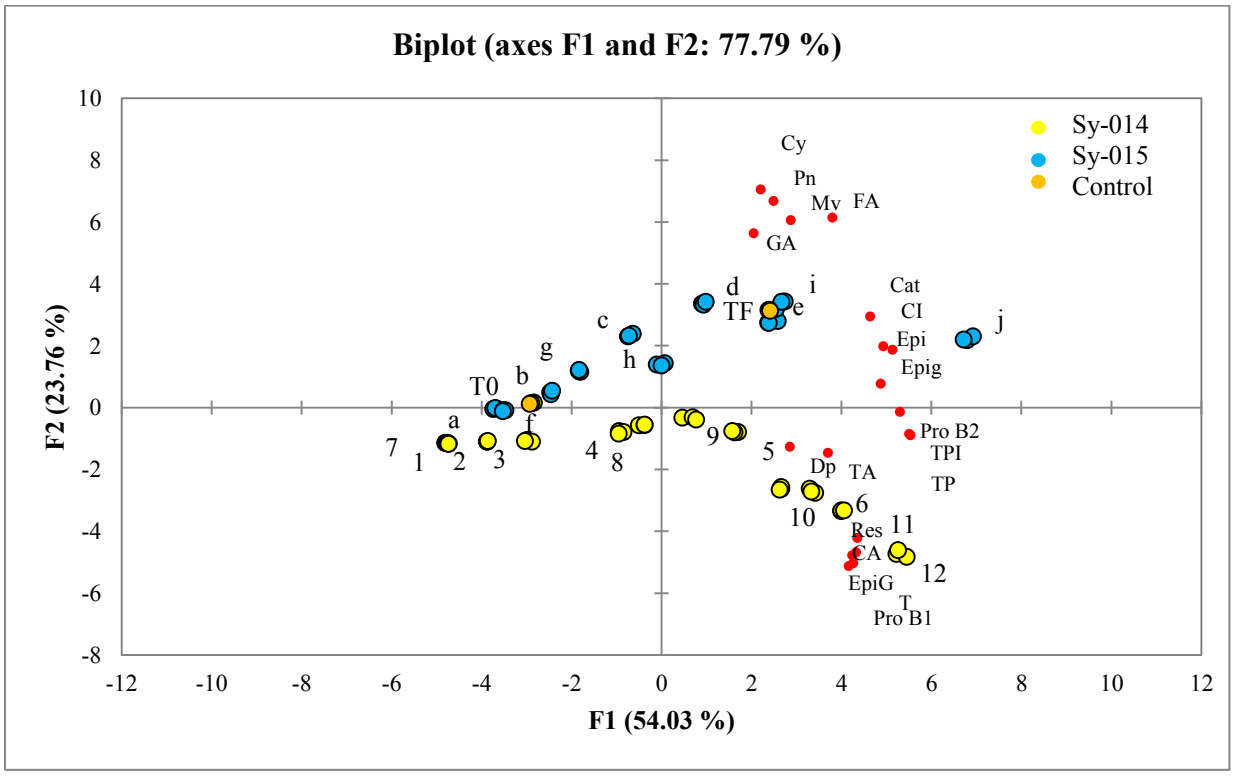

Figure II.2.3-a: Biplot of the two first principal components obtained from the colour and phenolic composition of 2014 and 2015 syrah vintages: TA, total anthocyanin content; CI, color intensity; TPI, total polyphenol index; TP, total polyphenols; T, Tannins; Dp, delphinidin-3-O-glucoside ; Cy, cyanidin-3-O-glucoside ; Pn, peonidin-3-O-glucoside ; Mv, malvidin-3-O-glucoside ; GA, gallic acid; pro B1, procyanidin B1; EpiG, epigallocatechin; cat, catechin; Pro B2, procyanidin B2; CA, caffeic acid; Epi, epicatechin; Epig, epicatechin gallate; FA, ferulic acid; Res; resveratrol; obtained after maceration at different temperatures for 48 and 24 hours respectively for 2014 and 2015 vintage (1, Sy-0-60°C-014 ; 2, Sy-2-60°C-014 ; 3, Sy-4-60°C-014 ; 4, Sy-8-60°C-014 ; 5, Sy-24-60°C-014; 6, Sy-48-60°C-014; 7, Sy-0-70°C-014 ; 8, Sy-2-70°C-014 ; 9, Sy-4-70°C-014 ; 10, Sy-8-70°C-014 ; 11, Sy-24-70°C-014; 12, Sy-48-70°C-014; a, Sy-0-60°C-015; b, Sy-2-60°C-015; c, Sy-4-60°C-015; d, Sy-8-60°C-015; e, Sy-24-60°C-015; f, Sy-0-70°C-015; g, Sy-2-70°C-015; h, Sy-4-70°C-015; i, Sy-8-70°C-015; j, Sy-24-70°C-015; T0, Syrah control at the beginning of maceration; TF, Syrah control at the end of fermentation.

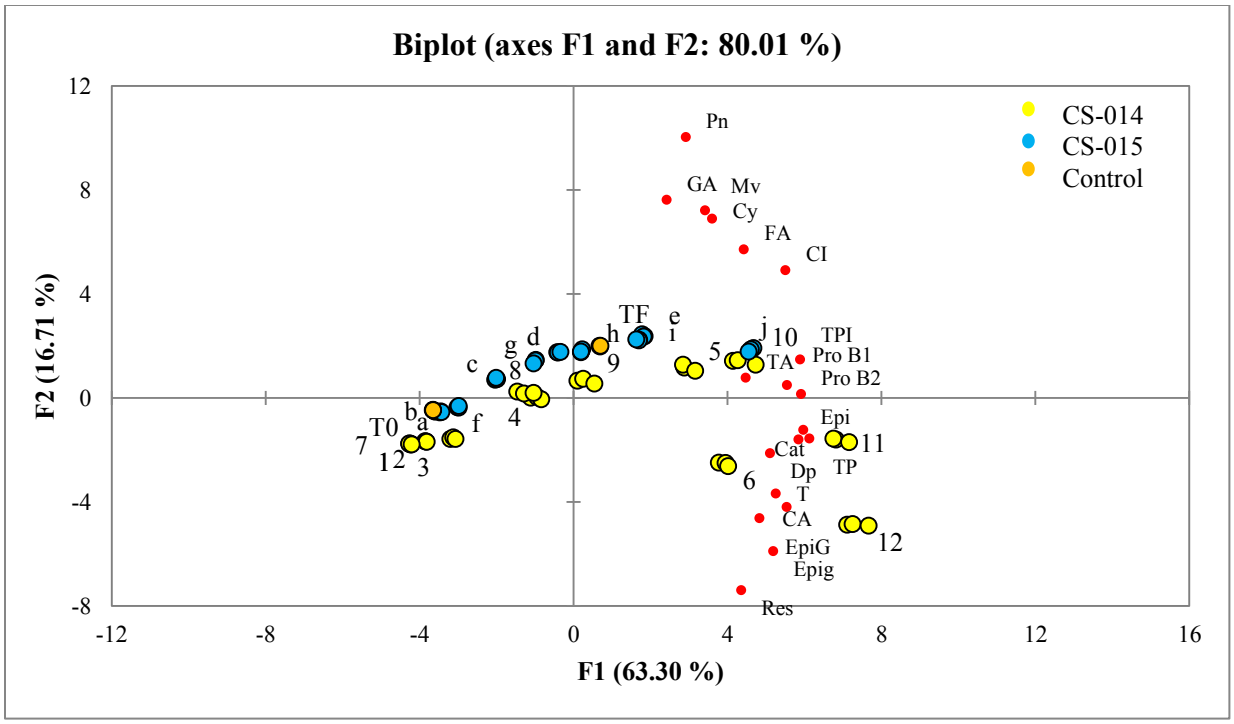

Figure II.2.3-b: Biplot of the two first principal components obtained from the colour and phenolic composition of 2014 and 2015 Cabernet Sauvignon vintages: TA, total anthocyanin content; CI, color intensity; TPI, total polyphenol index; TP, total polyphenols; T, Tannins; ABTS, Dp, delphinidin-3-O-glucoside ; Cy, cyanidin-3-O-glucoside ; Pn, peonidin-3-O-glucoside ; Mv, malvidin-3-O-glucoside ; GA, gallic acid; pro B1, procyanidin B1; EpiG, epigallocatechin; cat, catechin; Pro B2, procyanidin B2; CA, caffeic acid; Epi, epicatechin; Epig, epicatechin gallate; FA, ferulic acid; Res; resveratrol; obtained after maceration at different temperatures for 48 and 24 hours respectively for 2014 and 2015 vintage (1, CS-0-60°C-014 ; 2, CS-2-2-60°C-014 ; 3, CS-4-60°C-014 ; 4, CS-8-60°C-014 ; 5, CS-24-60°C-014; 6, CS-48-60°C-014; 7, CS-0-70°C-014 ; 8, CS-2-70°C-014 ; 9, CS-4-70°C-014 ; 10, CS-8-70°C-014 ; 11, CS-24-70°C-014; 12, CS-48-70°C-014; a, CS-0-60°C-015; b, CS-2-60°C-015; c, CS-4-60°C-015; d, CS-8-60°C-015; e, CS-24-60°C-015; f, CS-0-70°C-015; g, CS-2-70°C-015; h, CS-4-70°C-015; i, CS-8-70°C-015; j, CS-24-70°C-015; T0, Cabernet Sauvignon control at the beginning of maceration; TF, Cabernet Sauvignon control at the end of fermentation

II.2.5. Conclusion

In this work, we demonstrated that total anthocyanin content increases with temperature and maceration time up to a certain limit while the extraction of tannins is progressive over time. Extraction of total anthocyanins and tannins were favored by the pectolytic enzyme addition. Analyses of biological activities showed that must macerated for 48 hours presented higher percentage and different types of biological activities compared to must macerated for 24 hours. Results from PCA showed that vintage effect was observed on each studied phenolic compound concentrations and was more important for Syrah than Cabernet Sauvignon. At the end, due to some particular weather conditions, 2014 vintage of the two grape varieties showed higher total polyphenol content than 2015.

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Chapter III. Effect of Alcoholic fermentation

III.1. Introduction

Phenolic compounds of wine contribute to its sensorial properties such as color, bitterness, astringency and mouthfeel (Boulton, 2001; Vidal et al., 2004). These phenolic substances are extracted from the seeds, skins and stems of grapes during the maceration and fermentation processes. According to several epidemiological, clinical and in vitro studies, these compounds reduce the risk of various degenerative diseases (cardiovascular diseases, cancer, neurodegenerative diseases, diabetes and osteoporosis) due to their antioxidant activity (Scalbert et al., 2005; Stoclet at al., 2004). Wine phenolic contents depend on grape variety, vintage and winemaking conditions. Several studies have been published on those winemaking conditions that may promote greater extraction of phenolics and stable colour: length of maceration (Baustita-ortín et al., 2004; Gómez-Plaza et al., 2001; Vrhovsek et al., 2002), different maceration techniques (Moutounet et al., 2000; Netzel et al., 2003; Sun et al., 2001; Gordillo et al., 2010), the use of macerating enzymes (Canal-Llaubères and Pouns, 2002; Baustita-Ortín et al., 2007; Kammerer et al., 2005; Pardo et al., 1999; Busse-Valverde et al., 2011), the addition of oenological tannins (Zamora, 2003; Celotti et al., 2000). In addition to classical enological parameters, the selection of yeast strain has been shown to impact on the concentration of anthocyanins (Monagas et al., 2007; Morata et al., 2006) and others phenolics (Barcenila et al., 1989; Sidari et al., 2001; Monagas et al., 2007; Torrens et al., 2008) in finished wine. An interesting correlation between yeast strains and the phenolic composition of wines has been reported previously (Caridi et al., 2004), indicating that strain-dependent modification could significantly influence the colour properties, phenolic profile and antioxidant power of wines. Yeasts have different capacities to retain or adsorb phenolic compounds via van der Waals bonds and H-bonds (Vasserot et al., 1997; Morata et al., 2005). Also, other factors will affect adsorption, such as temperature, ethanol content, and the SO_2 present in the wine (Vasserot et al., 1997). Moreover, some yeast strains may express β-glucosidase activities promoting anthocyanins degradation, resulting from the breakdown of the glucosidic bond of the anthocyanidin-3-glucoside, these latter forms are less stable and could easily be degraded during wine ageing (Hernández et al., 2003). On the other hand, yeast may contribute to stabilizing wine colour as a result of participating in the formation of anthocyanins derivatives such as vistin A, vistin B and ethyl-linked anthocyanin-flavanol pigments (formed by the reaction between anthocyanins and secondary metabolites produced during yeast fermentation such as pyruvic

acid and acetaldehyde) (Escribano-Bailón et al., 2001; Bakker and Timberlake; 1997). These pigments exhibit a red-orange colour and are more resistant to pH changes and SO_2 bleaching than monomeric anthocyanins (Bakker and Timberlake, 1997; Fulcrand et al., 1998). Moreover, yeast may also liberate mannoproteins that have capacity to bind to anthocyanins and tannins (Escot et al., 2001) diminishing their reactivity and protecting them from precipitation. Today, a wide range of wine yeast strains are commercially available, which offers winemaking the opportunity to explore one or more suitable yeasts to assure a rapid and reliable fermentation process, and give wines a consistent and predictable quality (Rodriguez et al., 2010).

In this context the purpose of this study was to elucidate the effect of two different commercial yeast strains on wine colour, phenolic compounds and biological activities from must of two grape varieties (Syrah and Cabernet Sauvignon) from two distinct regions (Saint Thomas and Florentine), premacerated at different temperatures, with or without adding enzymes, As well as the effect of maceration enzymes on polyphenol composition of wines after alcoholic fermentation of Syrah and Cabernet Sauvignon Saint Thomas from the 2015 vintage premacerated at different temperatures with and without added enzymes (70°C, 70°C + enzymes) compared to the control fermented by X and Y strains with and without enzymes.

III.2. Materials and methods

III.2.1. CHEMICALS, CULTURE MEDIA AND STANDARDS

All chemicals used were of analytical reagent grade. All chromatographic solvents were high-performance liquid chromatography (HPLC) grade. All reagents and culture media were purchased from Sigma-Aldrich (France and Germany). All phenolic standards were obtained from Extrasynthese (Genay, France).

III.2.2. STRAINS AND STORAGE CONDITIONS

S. cerevisiae X and Y used in this work were kindly provided by Lallemand Inc. (Blagnac, France). X strain promotes qualitative potential and aromatic expression of Bordeaux wine regions while Y strain enhances varietal aromas for Bourgogne wine regions. Yeast stock cultures were kept at 4°C in YEPD (Yeast Extract Peptone Dextrose) agar slants composed of 10 g/l Yeast Extract, 20 g/l peptone, 20 g/l D-glucose and 20 g/l agar. The yeast inoculum was prior prepared in two steps. First, a preculture of the yeast strain was obtained by reactivating the stock

culture in YEPD broth for 24 h. Second, the preculture was used to inoculate a low sugar concentration synthetic grape juice medium composed of 50 g/l D-Glucose, 1 g/l Yeast extract, 2 g/l Ammonium sulfate, 0.3 g/l Citric acid, 5 g/l L-malic acid, 5 g/l L-tartaric acid, 0.4 g/l Magnesium sulfate and 5 g/l Potassium dihydrogen phosphate. This step was carried out for 24 h and provided the yeast inoculum.

III.2.3. VINIFICATIONS

The experiments were developed in two harvesting seasons, 2014 and 2015. Red grapes of *Vitis vinifera* var. Cabernet Sauvignon (CS) and Syrah (Sy) were supplied by two distinct regions Chateau St Thomas (West Bekaa / Lebanon) and Chateau Florentine (Chouf District / Lebanon) for the 2014 vintage and from one region (Chateau St Thomas) for the 2015 vintage. Grapes were harvested in 2014 and 2015 at optimum maturity into 20 kg boxes and transported to the laboratory. The grapes were crushed and destemmed manually and sodium metabisulphite was added (5 g of $NaHSO_3$/100 kg). 2 kg of grapes were transfered into glass Erlenmeyer flasks of 2 L and the pre-fermentative maceration was conducted at different temperatures (10, 60, 70 and 80°C) for 48 hours for the 2014 vintage and at temperatures of (60, 70 and 70°C + enzyme) for 24 hours for the 2015 vintage. Commercial pectolytic enzymes (5 g/100 kg grapes, LAFASE HE Grand Cru), were added 2 hours (at room temperature) prior to maceration at 70°C. Classical winemaking process of Syrah and Cabernet Sauvignon Saint Thomas for the 2015 harvest (maceration and fermentation occurs together at 25°C) with or without added enzymes were used as control. The Total acidity and pH of the two grape varieties (Syrah and Cabernet Sauvignon) musts from the two distinct regions (Saint Thomas) and the two vintages (2014 and 2015) are respectively presented in Table III.1, III.2 and III.3. At the end of the prefermentive maceration of musts at different temperatures, the pomace was pressed off and yeasts were added. Musts issued from different prefermentive temperatures were separately inoculated by two different yeasts strains *S. cerevisiae* X and *S. cerevisiae* Y at an initial concentration of 3×10^6 cells/ml (Thoma counting chamber). The AF was followed until total cessation of sugar consumption (< 2 g/l, DNS colorimetric method Miller, 1959). For both strains, the duration of AF was 10 days. At the end of this period the pomace was pressed off (for the control), yeast cells and enzymes were removed by centrifugation (3000 rpm for 15 min at 4°C) and sodium metabisulfite (50 mg/l) was

added. Wine samples were stored at 2°C until analysis. All fermentations were carried out in triplicate.

Table III.1: Characteristics of Y and X fermented wines (end of fermentation) from *Vitis vinifera* L. cv. Syrah and Cabernet Sauvignon Saint Thomas from 2014 vintage premacerated at different temperatures (10°C, 60°C,70°C and 80°C)

	Syrah saint Thomas-2014		Cabernet Sauvignon Saint Thomas-2014	
	Total acidity (g/L sulfuric acid)	pH	Total acidity (g/L sulfuric acid)	pH
10°C-Y	3.87 ± 0.07	3.74 ± 0.01	4.02 ± 0.14	3.94 ± 0.04
60°C-Y	4.46 ± 0.07	3.70 ± 0.06	5.29 ± 0.14	3.82 ± 0.06
70°C-Y	4.41 ± 0.00	3.76 ± 0.02	5.59 ± 0.14	3.92 ± 0.00
80°C-Y	4.85 ± 0.06	3.73 ± 0.06	4.30 ± 0.55	3.93 ± 0.01
10°C-X	4.95 ± 0.14	3.83 ± 0.02	5.43 ± 0.14	3.84 ± 0.01
60°C-X	4.21 ± 0.00	3.72 ± 0.07	5.83 ± 0.21	3.93 ± 0.01
70°C-X	5.92 ± 0.03	3.76 ± 0.07	5.63 ± 0.07	3.85 ± 0.02
80°C-X	6.12 ± 0.03	3.65 ± 0.07	4.10 ± 0.14	3.95 ± 0.01

Mean (n =3) ± SD

Table III.2: Characteristics of Y and X fermented wines (end of fermentation) from *Vitis vinifera* L. cv. Syrah and Cabernet Sauvignon Florentine from 2014 vintage premacerated at different temperatures (10°C, 60°C, 70°C and 80°C)

	Syrah Florentine-2014		Cabernet Sauvignon Florentine-2014	
	Total acidity (g/L sulfuric acid)	pH	Total acidity (g/L sulfuric acid)	pH
10°C-Y	4.12 ± 0.00	3.79 ± 0.05	5.19 ± 0.14	3.80 ± 0.03
60°C-Y	5.04 ± 0.07	3.90 ± 0.00	5.63 ± 0.21	3.77 ± 0.01
70°C-Y	4.63 ± 1.20	3.84 ± 0.03	5.19 ± 0.41	3.87 ± 0.02
80°C-Y	-	-	4.94 ± 0.06	3.88 ± 0.06
10°C-X	4.02 ± 0.14	3.77 ± 0.01	5.01 ± 0.72	3.82 ± 0.08
60°C-X	4.26 ± 0.07	3.91 ± 0.04	5.29 ± 0.35	3.61 ± 0.18
70°C-X	4.30 ± 0.21	3.86 ± 0.02	4.11 ± 0.07	3.96 ± 0.01
80°C-X	-	-	4.85 ± 0.04	4.00 ± 0.01

Mean (n =3) ± SD

Table III.3: Characteristics of Y and X fermented wines (end of fermentation) from *Vitis vinifera* L. cv. Syrah and Cabernet Sauvignon Saint Thomas from 2015 vintage premaceraated at different temperatures with or without added enzymes (60°C, 70°C and 70°C + enzymes, end of maceration) compared to control wines (25°C and 25°C + enzymes, end of maceration)

	Syrah Saint Thomas-2015		Cabernet Sauvignon Saint Thomas-2015	
	Total acidity (g/L sulfuric acid)	pH	Total acidity (g/L sulfuric acid)	pH
60°C-Y	4.67 ± 0.06	3.26 ± 0.01	4.28 ± 1.00	3.34 ± 0.03
70°C-Y	4.74 ± 0.06	3.33 ± 0.01	4.04 ± 0.28	3.34 ± 0.03
70°C + enzymes-Y	3.72 ± 0.05	3.38 ± 0.01	4.79 ± 0.79	3.25 ± 0.02
25°C-Y	4.77 ± 0.25	3.51 ± 0.03	4.21 ± 0.00	3.50 ± 0.01
25°C + enzymes-Y	4.28 ± 0.15	3.56 ± 0.01	4.60 ± 0.00	3.52 ± 0.05
60°C-X	4.77 ± 0.04	3.18 ± 0.01	4.87± 0.20	3.32 ± 0.01
70°C-X	4.70 ± 0.10	3.28 ± 0.03	4.82 ± 0.23	3.32 ± 0.00
70°C + enzymes-X	3.85 ± 0.06	3.34 ± 0.03	4.99 ± 0.23	3.30 ± 0.01
25°C-X	4.51 ± 0.15	3.51 ± 0.02	3.92 ± 0.00	3.52 ± 0.02
25°C + enzymes-X	4.60 ± 0.20	3.50 ± 0.02	4.02 ± 0.00	3.50 ± 0.00

Mean (n =3) ± SD

III.2.4. ANALYTICAL METHOD

Titratable acidity (expressed as g/L of sulfuric acid) and pH were determined according to the official methods of (OIV, 2005) at the beginning and the end of alcoholic fermentation.

III.2.5. SPECTROPHOTOMETRIC DETERMINATIONS (see II.1.2.5. p. 88)

III.2.6. HPLC ANALYSES OF PHENOLIC COMPOUNDS (see II.1.2.6. p. 89)

III.2.7. DETERMINATION OF BIOLOGICAL ACTIVITIES (see II.1.2.7. p. 89-93)

III.3. Results and discussion

III.3.1. GRAPE VARIETIES

III.3.1.1 Spectrophotometric analyses of polyphenols

The total anthocyanin, the phenolic profile and the antioxidant activity of Syrah and Cabernet Sauvignon wines from two distinct regions resulting from the alcoholic fermentation of musts macerated at different temperatures were reported in Table III.4 and III.5. As observed in Table III.4 and III.5, the wines premacerated at 60°C showed high total anthocyanin content. A wide

range of total anthocyanins concentrations was revealed, varying from 135 (Sy-F) to 403 mg/l (CS-F), with an average amount of 272.79 mg/l. Different behaviors are observed depending on grape variety and yeast strain. Cabernet Sauvignon wines showed higher amounts of total anthocyanins than Syrah. Also, wines fermented by Y strain presented higher concentrations of anthocyanins compared to those fermented by X strain. Contrariwise, wines macerated at 70°C showed higher TPI, total polyphenol content, tannins and antioxidant activities. In fact, the release of tannins requires longer maceration times and high temperatures (Guerrero et al., 2009), which indicates that the duration of contact between pomace and juice is an important factor for the extraction of polyphenols in wine. As seen in Tables III.4 and III.5, strain X compared to strain Y produced a wine with significantly higher average values of TPI, phenol content and tannins (83.87; 4290.33 mg/l and 4732.76 mg/l respectively), Cabernet Sauvignon Florentine presented the higher concentrations. All wines tested in this study showed an evident antioxidant activity (IC50 ranges between 0.01 and 2.25 mg/ml). Although strain X had the highest phenolic content, Y strain with few exceptions showed the highest antioxidant activity (lowest IC50 value) which indicated that not all phenolic compounds have the same contribution to the antioxidant activities (Rice-Evans et al., 1997). The higher content in ferulic and caffeic acids, procyanidin B1 and B2 and epicatechin in wines fermented by strain Y could be the most responsible for the antioxidant activity. During our experiment, it was found that a detectable maximum drop of almost 38.89% (Sy-ST, 60°C), 40.36% (CS-ST-60°C), 39.57% (Sy-F-10°C) and 38.49% (CS-F-70°C) in total anthocyanin. A maximum drop of almost 44.92% (Sy-ST-10°C), 42.24% (CS-ST-10°C), 24.91% (Sy-F-10°C) and 18.07% (CS-F, 60°C) in total phenolic content was recorded after alcoholic fermentation, probably due to the adsorption of phenolics onto yeast cells and the reaction with cell wall proteins but also the reactions of anthocyanins with other wine components (Czyzowska and Pogorzelski, 2002).

Table III.4: Total anthocyanin, phenolic profile, and antioxidant activity in wines from *Vitis vinifera* L. cv. Syrah and Cabernet Sauvignon Saint Thomas of 2014 vintage, resulting from the alcoholic fermentation of the must premacerated at different temperatures (10°C, 60°C, 70°C and 80°C) with two different yeast strains

		Sy-ST-2014			CS-ST-2014		
			Y	X		Y	X
		T0	TF	TF	T0	TF	TF
10°C	TA	50.46 ± 0.50	47.51 ± 0.22^{a}	31.71 ± 0.68^{b}	78.44 ± 0.49	69.12 ± 0.00^{a}	65.62 ± 2.65^{b}
	TPI	10.033 ± 0.56	5.07 ± 0.06^{b}	8.00 ± 0.00^{a}	15.70 ± 0.00	13.57 ± 0.11^{b}	15.60 ± 0.17^{a}
	TP	656.67 ± 5.77	361.67 ± 2.89^{b}	398.33 ± 2.89^{a}	891.67 ± 2.89	515.00 ± 13.23^{b}	851.67 ± 2.89^{a}
	T	634.45 ± 2.98	231.96 ± 0.00^{b}	303.08 ± 5.37^{a}	832.38 ± 3.87	367.40 ± 0.48^{b}	502.55 ± 0.05^{a}
	ABTS	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
60°C	TA	329.55 ± 5.02	235.25 ± 0.58^{a}	201.36 ± 11.30^{b}	571.57 ± 2.87	346.06 ± 2.87^{a}	340.87 ± 3.03^{b}
	TPI	59.13 ± 0.23	51.27 ± 3.82^{a}	57.20 ± 1.93^{a}	68.71 ± 1.70	56.80 ± 0.18^{b}	65.29 ± 0.27^{a}
	TP	3133.33 ± 28.87	2450.33 ± 0.57^{b}	2760.00 ± 35^{a}	3968.33 ± 10.40	2638.33 ± 16.07^{b}	2925.00 ± 5.00^{a}
	T	6492.82 ± 1.80	2064.87 ± 2.98^{b}	2362.47 ± 13.10^{a}	7477.29 ± 1.58	3382.55 ± 0.36^{b}	3955.20 ± 23.24^{a}
	ABTS	2.16 ± 0.07	5.50 ± 0.00^{b}	6.20 ± 0.00^{a}	2.35 ± 0.02	3.25 ± 0.00^{a}	2.90 ± 0.00^{b}
70°C	TA	141.46 ± 2.52	118.17 ± 4.99^{a}	98.81 ± 2.54^{b}	259.21 ± 4.09	239.66 ± 1.80^{a}	235.25 ± 1.52^{b}
	TPI	83.13 ± 2.37	73.17 ± 0.58^{b}	80.37 ± 0.46^{a}	87.45 ± 0.35	77.97 ± 1.08^{b}	80.13 ± 3.26^{a}
	TP	4146.67 ± 11.54	3101.67 ± 2.89^{b}	3761.33 ± 5.50^{a}	4000.67 ± 0.58	3723.33 ± 15.27^{b}	3976.67 ± 7.64^{a}
	T	7616.84 ± 1.66	3896.58 ± 1.93^{a}	4000.66 ± 0.56^{a}	10551.27	4245.05 ± 6.66^{b}	4400.62 ± 136.83^{a}
	ABTS	1.97 ± 0.06	3.00 ± 0.29^{b}	4.95 ± 0.06^{a}	2.00 ± 0.00	2.25 ± 0.05^{a}	2.52 ± 0.13^{a}
80°C	TA	47.54 ± 2.02	36.84 ± 2.30^{a}	34.08 ± 4.54^{b}	61.25 ± 0.00	50.51 ± 1.00^{a}	49.89 ± 1.43^{a}
	TPI	74.92 ± 2.75	54.50 ± 0.87^{b}	57.40 ± 0.78^{a}	85.53 ± 0.12	63.53 ± 0.47^{b}	65.77 ± 0.41^{a}
	TP	3211.67 ± 2.89	2295.33 ± 5.77^{b}	2585.67 ± 24.70^{a}	3215.67 ± 2.89	2978.33 ± 7.64^{b}	3076.74 ± 7.61^{a}
	T	4091.84 ± 122.48	1845.90 ± 9.33^{b}	3111.53 ± 0.93^{a}	6079.52 ± 19.17	2858.52 ± 14.70^{b}	3159.74 ± 10.61^{a}
	ABTS	3.12 ± 0.11	4.75 ± 0.27^{a}	4.15 ± 0.11^{b}	2.42 ± 0.14	4.01 ± 0.02^{b}	5.13 ± 0.06^{a}

Mean (n =3) ± SD. For each yeast strain from the same varietal, different letters in the same row indicate significant difference at $p < 0.05$. TA, total anthocyanin; TPI, total phenolic index, TP, total phenolic; T, Tannins

Table III.5: Total anthocyanin, phenolic profile, and antioxidant activity in wines from *Vitis vinifera* L. cv. Syrah and Cabernet Sauvignon Florentine of 2014 vintage, resulting from the alcoholic fermentation of the must premacerated at different temperatures (10°C, 60°C and 70°C) with two different yeast strains

		Sy-F-2014			CS-F-2014		
			Y	X		Y	X
		T0	TF	TF	T0	TF	TF
10°C	TA	65.92 ± 2.02	59.75 ± 0.21^{a}	39.83 ± 2.74^{b}	80.88 ± 1.02	68.02 ± 2.22^{a}	65.70 ± 0.70^{a}
	TPI	17.17 ± 0.23	10.74 ± 0.12^{a}	10.97 ± 0.47^{a}	24.63 ± 1.15	15.53 ± 0.06^{a}	13.61 ± 0.15^{a}
	TP	475.00 ± 5.00	356.67 ± 2.89^{b}	408.33 ± 2.89^{a}	1025.67 ± 6.03	861.67 ± 2.89^{b}	1006.33 ± 5.50^{a}
	T	2377.16 ± 3.00	205.65 ± 0.00^{b}	233.86 ± 1.85^{a}	947.40 ± 2.28	489.93 ± 0.03^{b}	917.69 ± 2.68^{a}
	ABTS	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
60°C	TA	170.36 ± 3.98	154.08 ± 6.12^{a}	135.20 ± 6.79^{b}	574.71 ± 17.54	403.96 ± 1.01^{a}	365.57 ± 0.28^{b}
	TPI	69.90 ± 1.61	62.37 ± 0.55^{b}	70.13 ± 0.06^{a}	71.45 ± 0.45	67.67 ± 0.11^{b}	70.40 ± 0.17^{a}
	TP	3465 ± 0.63	2817.33 ± 2.52^{b}	2993.33 ± 2.89^{a}	4093.33 ± 2.89	3353.33 ± 5.77^{b}	3476.67 ± 2.89^{a}
	T	7962.83 ± 0.33	2576.03 ± 12.44^{b}	3489.04 ± 7.18^{a}	10349.91 ± 25.89	3593.69 ± 2.60^{b}	4434.28 ± 11.15^{a}
	ABTS	3.50 ± 0.00	3.83 ± 0.06^{a}	4.00 ± 0.00^{a}	1.73 ± 0.05	3.30 ± 0.00^{a}	3.50 ± 0.00^{b}
70°C	TA	76.96 ± 2.36	74.96 ± 1.34^{a}	64.46 ± 1.01^{b}	298.11 ± 0.53	226.92 ± 11.11^{a}	183.37 ± 2.19^{b}
	TPI	85.23 ± 0.40	77.10 ± 0.87^{b}	80.67 ± 0.90^{a}	90.60 ± 0.17	80.40 ± 0.00^{b}	83.87 ± 0.81^{a}
	TP	3548.33 ± 2.89	2895.67 ± 1.15^{b}	3058.33 ± 2.87^{a}	4750.00 ± 30.00	3378.67 ± 105.40^{b}	4290.33 ± 93.05^{a}
	T	9176.91 ± 133.38	3557.38 ± 5.74^{b}	4280.30 ± 15.43^{a}	11734.91 ± 0.98	4656.96 ± 2.70^{b}	4732.76 ± 10.94^{a}
	ABTS	1.32 ± 0.01	3.65 ± 0.00^{b}	4.20 ± 0.00^{a}	3.95 ± 0.17	3.00 ± 0.06^{a}	2.50 ± 0.06^{b}
80°C	TA	-	-	-	72.04 ± 2.78	68.75 ± 0.08^{a}	59.96 ± 0.4^{b}
	TPI	-	-	-	89.60 ± 0.17	64.07 ± 1.41^{a}	70.30 ± 0.29^{a}
	TP	-	-	-	3555.00 ± 104.49	3042.00 ± 42.64^{a}	3173.33 ± 52.89^{a}
	T	-	-	-	8244.83 ± 0.81	2958.99 ± 44.31^{b}	3575.51 ± 87.42^{a}
	ABTS	-	-	-	3.10 ± 0.06	4.42 ± 0.06^{a}	4.40 ± 0.17^{a}

Mean (n =3) ± SD. For each yeast strain, different letters in the same row indicate significant difference at $p < 0.05$. TA, total anthocyanin; TPI, total phenolic index, TP, total phenolic; T. Tannins.

III.3.1.2. HPLC analyses of polyphenols

III.3.1.2.1 Anthocyanins

Table III.6 and III.7 summarizes the individual anthocyanin concentration in wines from *V. vinifera* L. cv. Syrah and Cabernet Sauvignon from two distinct regions, resulting from the alcoholic fermentation of the must macerated at different temperatures with the two yeast strains. As it can be seen from Table III.6 and III.7, the must macerated at 60°C for 48 hours, with few exceptions presented higher content in monomeric anthocyanins (66.8 and 80.98 mg/l for Sy and CS Saint Thomas respectively; 15.84 and 148.75 mg/l for Sy and CS Florentine respectively), followed by the must macerated at 10, 70 and 80°C. This decrease in monomeric anthocyanins could be explained by the fact that anthocyanins are highly sensitive compounds which are degraded at high temperatures (Galvin 1993).

Our results showed (Table III.6 and III.7) that malvidin-3-O-glucoside was the major anthocyanin composing 38.33-78.09% (Sy-ST), 32.44-81.88% (CS-ST), 41.99-66.33% (Sy-F) and 34.67-81.91% (CS-F) of total anthocyanins quantified by HPLC after alcoholic fermentation in accordance with many authors (Núñez et al., 2004; Figuèiredo-González et al., 2012). On the other hand, cyanidin-3-O-glucoside showed the low concentration (n.d-1.79 mg/l, Sy and CS-ST) and (n.d-3.15 mg/l, Sy and CS-F), probably because this anthocyanin is the precursor of all others (Núñez et al., 2004). The Syrah fermented wine by Y strain from the two distinct regions, showed a significantly higher anthocyanin concentration than the wines fermented by X strain (Table III.6 and III.7). A similar trend was observed for the Cabernet Sauvignon wines from the two different regions. However the content of individual anthocyanin differed significantly among the yeast strains, especially in the case of the major anthocyanins. In specific the amount of malvidin-3-O-glucoside was almost 1.82, 1.57 and 1.88 times higher for Sy-ST-Y fermented wines premacerated respectively at 10, 60 and 70°C than X strain for the same variety and maceration temperatures. Moreover, malvidin-3-O-glucoside mean values were 1.47; 1.20; 1.15 and 1.03 times higher for CS-Y fermented wines (from the two regions) than CS-X (from the two regions) fermented wines premacerated respectively at 10°C, 60°C, 70°C and 80°C.

Therefore, from the maceration step to the end of alcoholic fermentation, the changes in concentrations of anthocyanin compounds resulted in significantly decreased about 44.07; 64.92; 52.35 and 6.64% respectively for Sy-ST X fermented wines at temperatures of 10, 60, 70 and 80°C and 32.00; 59.41 and 53.63% respectively for Sy-F X fermented wines at temperatures of

10°C, 60°C and 70°C. In addition, anthocyanin percentage decreased about 40.45; 56.16; 27.50; 12.20% for CS-ST and 34.10; 64.04; 45.47 and 29.40% for CS-F X fermented wines for must premacerated respectively at temperatures of 10°C, 60°C, 70°C and 80°C. This decrease in the total anthocyanin content could be explained by hydrolysis of the glucosidic bond of anthocyanidin-3-O-glucoside due to the presence of β-glucosidase activity of certain strain of *S. cerevisiae*, adsorption to yeast cell walls and or the formation of other anthocyanin-derived pigments (Morata et al., 2005; Vasserot et al., 1997; Escribano-Bailón et al., 2001). At last, quantitatively and regarding the difference between varieties, Cabernet sauvignon variety showed the highest content of anthocyanins in all analyzed samples along the winemaking process, actually because anthocyanin profile of a given grape variety is closely linked to its genetic inheritance, although environmental factors may have influence on this profile (de Villiers et al., 2004).

Table III.6: Anthocyanin monomers concentrations (mg/l) in wines from *Vitis vinifera* L. cv. Syrah and Cabernet Sauvignon Saint Thomas of 2014 vintage resulting from the alcoholic fermentation of the must macerated at different temperatures (10°C, 60°C, 70°C and 80°C) with two different yeast strains

		Syrah-ST-2014			Cabernet Sauvignon -ST-2014		
			Y	X		Y	X
	Simple glucosides	T0	TF	TF	T0	TF	TF
10°C	Dp-3-glc	4.04 ± 0.07	3.87 ± 0.11^{a}	3.04 ± 0.03^{b}	3.66 ± 0.01	3.51 ± 0.10^{a}	3.21 ± 0.12^{b}
	Cy-3-glc	n.d	n.d	n.d	1.87 ± 0.02	1.79 ± 0.03^{a}	1.13 ± 0.02^{b}
	Pn-3-glc	0.93 ± 0.01	0.82 ± 0.04^{a}	0.76 ± 0.00^{a}	1.29 ± 0.00	n.d	0.73 ± 0.00^{a}
	Mv-3-glc	16.18 ± 0.46	14.59 ± 0.06^{a}	8.03 ± 0.04^{b}	20.84 ± 0.07	15.65 ± 0.05^{a}	11.40 ± 0.49^{b}
	ΣAnt-glc	21.15 ± 0.54	19.28 ± 0.21^{a}	11.83 ± 0.07^{b}	27.66 ± 0.10	20.95 ± 0.18^{a}	16.47 ± 0.63^{b}
60°C	Dp-3-glc	5.32 ± 0.04	5.30 ± 0.18^{a}	4.59 ± 0.12^{b}	5.64 ± 0.01	5.74 ± 0.01^{a}	5.47 ± 0.18^{b}
	Cy-3-glc	3.45 ± 0.08	n.d	n.d	n.d	n.d	n.d
	Pn-3-glc	7.73 ± 0.03	2.30 ± 0.07^{a}	1.60 ± 0.02^{b}	3.04 ± 0.00	0.95 ± 0.01^{a}	0.97 ± 0.00^{a}
	Mv-3-glc	50.38 ± 0.63	27.09 ± 0.24^{a}	17.27 ± 0.36^{b}	72.30 ± 0.18	30.22 ± 0.15^{a}	29.06 ± 0.14^{b}
	ΣAnt-glc	66.88 ± 0.78	34.69 ± 0.49^{a}	23.46 ± 0.5^{b}	80.98 ± 0.19	36.91 ± 0.17^{a}	35.50 ± 0.32^{b}
70°C	Dp-3-glc	4.75 ± 0.11	4.65 ± 0.02^{a}	4.46 ± 0.08^{b}	6.14 ± 0.48	6.05 ± 0.02^{a}	5.34 ± 0.44^{b}
	Cy-3-glc	2.54 ± 0.03	n.d	n.d	n.d	n.d	n.d
	Pn-3-glc	1.33 ± 0.01	1.07 ± 0.07^{a}	n.d	1.02 ± 0.03	0.82 ± 0.01^{b}	0.98 ± 0.02^{a}
	Mv-3-glc	7.31 ± 0.11	5.89 ± 0.01^{a}	3.13 ± 0.06^{b}	11.86 ± 0.05	8.34 ± 0.04^{a}	7.47 ± 0.27^{b}
	ΣAnt-glc	15.93 ± 0.26	11.61 ± 0.1^{a}	7.59 ± 0.14^{b}	19.02 ± 0.56	15.21 ± 0.07^{a}	13.79 ± 0.73^{b}
80°C	Dp-3-glc	3.62 ± 0.02	3.54 ± 0.11^{a}	3.32 ± 0.13^{b}	4.53 ± 0.01	4.40 ± 0.06^{a}	3.82 ± 0.40^{b}
	Cy-3-glc	n.d	n.d	n.d	n.d	n.d	n.d
	Pn-3-glc	n.d	n.d	n.d	n.d	n.d	n.d
	Mv-3-glc	2.40 ± 0.01	2.20 ± 0.01^{a}	2.30 ± 0.02^{a}	2.27 ± 0.00	2.21 ± 0.02^{a}	2.15 ± 0.00^{b}
	ΣAnt-glc	6.02 ± 0.02	5.74 ± 0.11^{a}	5.62 ± 0.13^{a}	6.80 ± 0.01	6.61 ± 0.06^{a}	5.97 ± 0.40^{b}

Mean (n =3) ± SD. For each yeast strain, different letters in the same row indicate significant difference at $p < 0.05$. Dp, delphinidin; Cy, cyanidin; Pn, peonidin; Mv, malvidin; glc, glucoside; Ant, anthocyanin; n.d, not detected values

Table III.7: Anthocyanin monomers concentrations (mg/l) in wines from *Vitis vinifera* L. cv. Syrah and Cabernet Sauvignon Florentine of 2014 vintage resulting from the alcoholic fermentation of the must macerated at different temperatures (10°C, 60°C, 70°C and 80°C) with two different yeast strains.

	compounds	Sy-F-2014			CS-F-2014		
			Y	X		Y	X
	Simple glucosides	T0	TF	TF	T0	TF	TF
10°C	Dp-3-glc	3.28 ± 0.03	3.15 ± 0.21^{a}	3.13 ± 0.20^{a}	4.90 ± 0.03	4.60 ± 0.04^{a}	4.58 ± 0.05^{a}
	Cy-3-glc	1.86 ± 0.00	1.85 ± 0.00^{a}	1.56 ± 0.11^{b}	n.d	n.d	n.d
	Pn-3-glc	2.57 ± 0.02	0.91 ± 0.02^{a}	0.84 ± 0.01^{b}	1.98 ± 0.07	1.06 ± 0.01^{a}	0.92 ± 0.02^{b}
	Mv-3-glc	13.93 ± 0.07	12.94 ± 0.43^{a}	9.14 ± 0.03^{b}	18.81 ± 0.06	18.10 ± 0.70^{a}	11.43 ± 0.32^{b}
	Σant-glc	22.25 ± 0.12	19.51 ± 0.66^{a}	15.13 ± 0.35^{b}	25.69 ± 0.16	23.76 ± 0.75^{a}	16.93 ± 0.39^{b}
60°C	Dp-3-glc	4.58 ± 0.15	4.29 ± 0.26^{a}	4.02 ± 0.31^{b}	6.97 ± 0.25	6.92 ± 0.28^{a}	6.90 ± 0.06^{a}
	Cy-3-glc	2.27 ± 0.07	n.d	n.d	3.68 ± 0.17	3.15 ± 0.01^{a}	2.95 ± 0.04^{b}
	Pn-3-glc	1.21 ± 0.01	0.77 ± 0.01^{a}	0.78 ± 0.00^{a}	9.58 ± 0.08	2.46 ± 0.02^{a}	2.35 ± 0.11^{a}
	Mv-3-glc	6.60 ± 0.10	4.81 ± 0.32^{a}	4.20 ± 0.07^{a}	128.52 ± 0.11	56.72 ± 0.47^{a}	41.29 ± 0.78^{b}
	Σant-glc	15.84 ± 0.33	7.27 ± 0.59^{a}	6.43 ± 0.38^{a}	148.75 ± 0.61	69.25 ± 0.78^{a}	53.49 ± 0.99^{b}
70°C	Dp-3-glc	3.78 ± 0.14	3.65 ± 0.03^{a}	3.62 ± 0.28^{b}	5.59 ± 0.01	5.46 ± 0.41^{a}	5.40 ± 1.53^{b}
	Cy-3-glc	n.d	n.d	n.d	1.43 ± 0.02	n.d	n.d
	Pn-3-glc	n.d	n.d	n.d	1.55 ± 0.03	0.81 ± 0.00^{a}	0.82 ± 0.03^{a}
	Mv-3-glc	2.17 ± 0.02	2.15 ± 0.00^{a}	2.13 ± 0.00^{b}	18.17 ± 0.05	9.96 ± 0.26^{a}	8.36 ± 0.52^{b}
	Σant-glc	10.05 ± 0.16	5.12 ± 0.03^{a}	4.66 ± 0.28^{b}	26.74 ± 0.11	16.23 ± 0.67^{a}	14.58 ± 2.08^{b}
80°C	Dp-3-glc	-	-	-	5.77 ± 0.30	4.24 ± 0.11^{a}	3.46 ± 0.04^{b}
	Cy-3-glc	-	-	-	n.d	n.d	n.d
	Pn-3-glc	-	-	-	n.d	n.d	n.d
	Mv-3-glc	-	-	-	2.19 ± 0.01	2.25 ± 0.00^{a}	2.16 ± 0.01^{b}
	Σant-glc	-	-	-	7.96 ± 0.31	6.49 ± 0.11^{a}	5.62 ± 0.05^{b}

Mean (n =3) ± SD. For each yeast strain, different letters in the same row indicate significant difference at $p < 0.05$. Dp, delphinidin; Cy, cyanidin; Pn, peonidin; Mv, malvidin; glc; glucoside; Ant, anthocyanin; n.d, not detected values

Table III.8 and III.9 showed the individual concentration of the different non-flavonoid (phenolic acids and stilbenes) and flavonoid (flavanols) phenolic compounds in wines from *V. vinifera* L. cv. Syrah and Cabernet Sauvignon from two distinct regions. CS-F premacerated at 70°C presented the highest content of total non-anthocyanins phenolic compounds with a concentration of 1097.49 mg/l. Epigallocatechin was the most abundant flavanol in Syrah and Cabernet sauvignon wines from the two distinct regions. From must to wine, we observed a significant drop in the content of most flavanol compounds where fermented wines by Y strain presented the higher decrease. In addition, some individual flavanols showed a significant increase, which is probably the consequence of the hydrolysis that suffers their polymeric and galloylated precursors during winemaking process. The increase observed in (+)- Catechin, (-)-Epicatechin, Procyanidin B1 and B2 could be a consequence of the hydrolysis from their galloylated precursors, like epigallocatechin gallate, epicatechin gallte and procyanidin dimer monogallate respectively (Lingua et al., 2016), which also justifies the increase observed in gallic acid from must to wine fermented by the two yeast strains. Among varieties, CS-F-X-70°C fermented wines showed the highest content in flavanols. Besides, as it can be seen from Table III.8 and III.9, musts macerated at high temperatures (70°C and 80°C) didn‟t show the presence of gallic acid (under detection limit) probably due to their heat-sensitive nature. However in wines, gallic acid contents increased significantly, where the most important concentration was found in wines premacerated at 70°C and 80°C. It seems that the increase of gallic acid content is yeast strain dependant where X strain increased significantly the amount of gallic acid in wines compared to Y strain. This can be due to the action of hydrolysis of galloylated precursors by esterase activities.

Hydrolysis of both caffeic and ferulic tartaric acid esters (Ginjom et al., 2011) during winemaking process resulted in an increase of free caffeic and ferulic acids contents in wines. It seems that this increase depends on the grape variety, maceration temperature and yeast strain.

Concerning *trans*-resveratrol, Syrah musts had the highest level of *trans*-resveratrol with a concentration of 3.91 and 4.82 mg/l respectively for Sy-ST-70°C and Sy-F-60°C (Table III.5 and III.6). As seen in Table III.5 and III.6 the levels of *trans*-resveratrol in analyzed wine samples did not follow a common trend for the different varieties and the different maceration temperatures. In case of wines fermented by X strain, we observed that the content of *trans*-resveratrol increased significantly ($p < 0.05$) in Sy and CS wines from the two different regions

with a maximum concentration of 9.21 (+ 298.70%) and 8.26 mg/l (+ 109.64%) respectively for CS-ST-70°C-X and CS-F-60°C-X fermented wines compared to the musts. In contrast, the *trans*-resveratrol content was significantly decreased in wines fermented by Y strain (-87.93 and – 3.62 %) in the two grape varieties from the two distinct regions, with few exceptions. In addition, wines premacerated at 10°C and 80°C showed small variations in the level of resveratrol for the two yeast strains. Increasing value of resveratrol after alcoholic fermentation was probably due to the hydrolysis of their glucosidic form trans-picéid or and *cis/trans* isomerization that have been observed to occur during winemaking process (Monagas et al., 2005b), while their decreasing value was probably due to their adsorption by yeast cell walls (Barcia et al., 2014b)

Finally, the total concentration of anthocyanins (Table III.4 and III.5) and non-anthocyanins compounds (Table III.8 and III.9) showed a decrease in concentration after alcoholic fermentation for both yeast strains, other authors (Bonilla et al., 2001) observed that yeast not only adsorb anthocyanins but other phenolic compounds. In addition, the total concentration of non-anthocyanin phenolic compounds revealed differences between the wines derived from the Y and X yeast strains. Contrary to the results found in relation to the anthocyanins from which Y strain showed the higher concentration, X strain showed higher concentration of total non-anthocyanin compound suggesting more β-glucosidase activity for X strain and also high hydrophilic parietal constituents.

Table III.8: Individual non-anthocyanin phenolic compounds (mg/l) in wines from Vitis vinifera cv. Syrah and Cabernet Sauvignon Saint Thomas of 2014 vintage resulting from the alcoholic fermentation of the must premacerated at different temperatures (10°C, 60°C, 70°C and 80°C) with two different yeast strains

	compounds	Sy-ST-2014			CS-ST-2014		
			Y	X		Y	X
		T0	TF	TF	T0	TF	TF
10°C	*Flavanols*						
	(+)- Cat	20.87 ± 0.09	11.49 ± 0.13^{b}	20.94 ± 0.60^{a}	29.00 ± 0.67	3.67 ± 0.07^{b}	6.18 ± 0.09^{a}
	(-)- Epi	24.05 ± 0.01	15.98 ± 0.02^{b}	24.00 ± 0.51^{a}	20.13 ± 0.01	17.43 ± 0.42^{b}	19.57 ± 0.21^{a}
	(-)- Epig	4.27 ± 0.00	2.08 ± 0.03^{b}	3.41 ± 0.11^{a}	6.04 ± 0.00	3.71 ± 0.04^{b}	4.17 ± 0.01^{a}
	(-)- EpiG	36.00 ± 0.59	23.72 ± 0.02^{b}	33.92 ± 0.53^{a}	56.23 ± 0.57	13.64 ± 0.30^{b}	43.17 ± 0.42^{a}
	Pro B1	11.33 ± 0.24	6.70 ± 0.20^{b}	11.19 ± 0.15^{a}	13.30 ± 0.43	7.55 ± 0.02^{b}	9.27 ± 0.12^{a}
	Pro B2	17.41 ± 0.01	8.51 ± 0.04^{b}	12.09 ± 0.05^{a}	13.85 ± 0.10	9.82 ± 0.23^{b}	7.94 ± 0.02^{a}
	Phenolic acids						
	Gallic acid	0.13 ± 0.00	0.13 ± 0.00^{a}	0.10 ± 0.01^{a}	0.17 ± 0.00	0.20 ± 0.00^{a}	0.17 ± 0.00^{a}
	Caffeic acid	2.27 ± 0.00	1.65 ± 0.04^{a}	1.69 ± 0.03^{a}	2.00 ± 0.00	1.26 ± 0.02^{b}	1.54 ± 0.02^{a}
	Ferulic acid	2.04 ± 0.00	1.41 ± 0.00^{b}	2.00 ± 0.05^{a}	2.47 ± 0.13	2.06 ± 0.08^{b}	2.45 ± 0.03^{a}
	Stilbenes						
	Resveratrol	0.61 ± 0.00	0.33 ± 0.00^{b}	1.23 ± 0.01^{a}	1.74 ± 0.01	0.21 ± 0.00^{b}	1.61 ± 0.00^{a}
	Total non-Ant	118.98 ± 0.94	72.00 ± 0.44^{b}	110.57 ± 2.05^{a}	144.93 ± 1.92	59.55 ± 1.18^{b}	96.07 ± 0.92^{a}
60°C	*Flavanols*						
	(+)- Cat	112.65 ± 1.39	38.98 ± 1.17^{b}	71.69 ± 0.56^{a}	143.30 ± 1.67	29.17 ± 0.66^{b}	100.84 ± 0.11^{a}
	(-)- Epi	92.28 ± 0.87	59.35 ± 2.00^{b}	88.30 ± 1.98^{a}	92.86 ± 0.88	73.35 ± 0.19^{b}	92.55 ± 0.16^{a}
	(-)- Epig	31.35 ± 0.32	26.57 ± 0.60^{b}	30.03 ± 0.98^{a}	48.75 ± 0.17	35.10 ± 0.36^{b}	45.10 ± 1.27^{a}
	(-)- EpiG	205.31 ± 1.13	177.18 ± 0.41^{b}	187.21 ± 0.14^{a}	259.10 ± 6.22	144.11 ± 1.20^{b}	239.87 ± 7.12^{a}
	Pro B1	82.94 ± 1.53	49.10 ± 0.00^{b}	51.91 ± 1.13^{a}	69.05 ± 1.04	36.29 ± 0.23^{b}	50.41 ± 2.50^{a}
	Pro B2	95.87 ± 0.89	80.28 ± 0.400^{b}	135.18 ± 3.03^{a}	84.94 ± 1.09	72.46 ± 0.35^{b}	85.25 ± 0.02^{a}
	Phenolic acids						
	Gallic acid	1.22 ± 0.02	0.88 ± 0.02^{b}	1.18 ± 0.00^{a}	1.71 ± 0.00	1.00 ± 0.00^{b}	1.44 ± 0.05^{a}
	Caffeic acid	5.21 ± 0.05	4.39 ± 0.02^{b}	4.61 ± 0.10^{a}	3.61 ± 0.17	2.53 ± 0.01^{b}	3.77 ± 0.02^{a}
	Ferulic acid	8.56 ± 0.08	15.14 ± 0.51^{a}	8.30 ± 0.06^{b}	7.18 ± 0.10	8.15 ± 0.03^{b}	10.40 ± 0.04^{a}
	Stilbenes						
	Resveratrol	3.02 ± 0.02	3.87 ± 0.05^{b}	6.77 ± 0.10^{a}	2.60 ± 0.04	2.42 ± 0.01^{b}	9.10 ± 0.03^{a}
	Total non-Ant	638.41 ± 6.28	455.74 ± 5.18^{b}	585.18 ± 8.08^{a}	713.10 ± 11.38	404.58 ± 3.04^{b}	638.73 ± 11.32^{a}

70°C	*Flavanols*						
	(+)- Cat	115.37 ± 0.41	103.74 ± 1.36^{a}	105.72 ± 1.22^{a}	119.41 ± 2.14	96.62 ± 1.56^{b}	116.24 ± 2.00^{a}
	(-)- Epi	156.54 ± 0.25	110.66 ± 0.35^{b}	133.42 ± 2.06^{a}	156.80 ± 2.69	109.32 ± 3.30^{b}	131.26 ± 2.27^{a}
	(-)- Epig	58.54 ± 0.05	57.76 ± 3.01^{b}	58.03 ± 0.03^{a}	58.31 ± 0.23	48.39 ± 0.32^{b}	56.08 ± 3.45^{a}
	(-)- EpiG	359.79 ± 0.40	213.82 ± 1.37^{b}	253.62 ± 3.63^{a}	404.84 ± 1.48	324.43 ± 3.25^{b}	340.85 ± 0.50^{a}
	Pro B1	108.17 ± 0.98	123.91 ± 0.44^{a}	65.52 ± 0.53^{b}	90.89 ± 0.52	65.75 ± 0.71^{b}	75.85 ± 1.17^{a}
	Pro B2	163.20 ± 2.27	126.08 ± 1.85^{b}	170.38 ± 1.38^{a}	169.30 ± 0.40	169.98 ± 0.57^{a}	152.67 ± 0.24^{b}
	Phenolic acids						
	Gallic acid	8.05 ± 0.23	6.33 ± 0.18^{a}	6.97 ± 0.18^{a}	5.60 ± 0.32	4.22 ± 0.08^{b}	5.15 ± 0.13^{a}
	Caffeic acid	8.66 ± 0.00	6.56 ± 0.11^{b}	7.63 ± 0.29^{a}	7.04 ± 0.02	5.93 ± 0.04^{b}	6.26 ± 0.05^{a}
	Ferulic acid	10.07 ± 0.09	19.08 ± 0.01^{a}	11.65 ± 0.22^{b}	8.88 ± 0.88	8.85 ± 0.91^{b}	9.35 ± 0.62^{a}
	Stilbenes						
	Resveratrol	3.91 ± 0.01	2.23 ± 0.02^{b}	6.36 ± 0.02^{a}	2.31 ± 0.08	2.10 ± 0.01^{b}	9.21 ± 0.26^{a}
	Total non-Ant	992.30 ± 4.66	770.17 ± 8.68^{b}	819.30 ± 9.56^{a}	1023.38 ± 8.87	835.59 ± 10.75^{b}	902.92 ± 10.69^{a}
80°C	*Flavanols*						
	(+)- Cat	92.99 ± 0.08	65.30 ± 0.50^{b}	111.88 ± 1.57^{a}	134.53 ± 0.48	126.44 ± 0.23^{b}	128.00 ± 0.52^{a}
	(-)- Epi	97.43 ± 0.65	73.33 ± 0.22^{b}	113.17 ± 1.74^{a}	116.02 ± 0.78	88.90 ± 0.90^{b}	98.73 ± 0.71^{a}
	(-)- Epig	37.15 ± 1.25	10.54 ± 0.06^{b}	32.52 ± 0.35^{a}	45.17 ± 0.88	35.12 ± 1.07^{b}	40.70 ± 0.62^{a}
	(-)- EpiG	304.83 ± 0.59	213.07 ± 3.31^{b}	283.79 ± 2.87^{a}	312.82 ± 0.89	275.64 ± 1.96^{b}	306.59 ± 0.16^{a}
	Pro B1	74.61 ± 3.39	44.93 ± 0.44^{b}	72.89 ± 1.34^{a}	84.35 ± 1.76	62.85 ± 0.45^{b}	68.15 ± 0.09^{a}
	Pro B2	113.13 ± 1.40	76.19 ± 1.41^{b}	158.58 ± 1.08^{a}	138.08 ± 0.05	130.09 ± 5.84^{a}	115.11 ± 1.29^{b}
	Phenolic acids						
	Gallic acid	19.75 ± 0.25	8.86 ± 0.34^{b}	13.07 ± 0.12^{a}	18.22 ± 0.49	14.53 ± 0.15^{b}	15.75 ± 0.28^{a}
	Caffeic acid	10.29 ± 0.26	7.07 ± 0.01^{b}	9.57 ± 0.01^{a}	9.17 ± 0.02	7.84 ± 0.25^{b}	8.73 ± 0.21^{a}
	Ferulic acid	13.60 ± 0.01	13.18 ± 0.04^{a}	13.35 ± 0.04^{a}	11.59 ± 0.18	11.02 ± 0.02^{a}	11.07 ± 0.48^{a}
	Stilbenes						
	Resveratrol	1.11 ± 0.01	1.44 ± 0.12^{b}	1.95 ± 0.04^{a}	1.38 ± 0.01	1.33 ± 0.01^{a}	1.63 ± 0.02^{a}
	Total non-Ant	764.89 ± 7.84	513.91 ± 6.45^{b}	808.82 ± 9.16^{a}	871.33 ± 5.43	753.76 ± 10.88^{b}	794.46 ± 5.38^{a}

Mean (n=3) ± SD. For each yeast strain, different letters in the same row indicate significant difference at $p < 0.05$. Cat, catechin; Epi, epicatechin; Epig, epicatechin gallate; EpiG, epigallocatechin; Pro B1, procyanidin B1; Pro B2, procyanidin B2; Ant, anth

Table III.9: Individual non-anthocyanin phenolic compounds (mg/l) in wines from *Vitis vinifera* cv. Syrah and Cabernet Sauvignon Florentine of 2014 vintage resulting from the alcoholic fermentation of the must premacerated at different temperatures (10°C, 60°C, 70°C and 80°C) with two different yeast strains

	compounds	**Sy-F-2014**			**CS-F-2014**		
			Y	X		Y	X
		T0	TF	TF	T0	TF	TF
10°C	*Flavanols*						
	(+)-Cat	13.16 ± 0.04	9.91 ± 0.01^{b}	11.86 ± 0.00^{a}	25.53 ± 0.25	7.59 ± 0.00^{b}	24.38 ± 1.08^{a}
	(-)- Epi	13.37 ± 0.69	13.26 ± 0.63^{b}	7.44 ± 1.63^{b}	27.54 ± 0.01	17.76 ± 0.34^{a}	21.13 ± 1.46^{a}
	(-)- Epig	5.10 ± 0.00	3.36 ± 0.01^{b}	4.71 ± 0.00^{a}	7.69 ± 0.24	4.63 ± 0.03^{b}	6.19 ± 0.22^{a}
	(-)- EpiG	82.75 ± 0.37	33.54 ± 0.08^{b}	39.58 ± 2.18^{a}	55.42 ± 2.74	46.77 ± 2.44^{b}	54.19 ± 0.09^{a}
	Pro B1	19.44 ± 0.47	11.22 ± 0.07^{b}	19.95 ± 0.77^{a}	14.56 ± 0.06	8.91 ± 0.01^{b}	13.51 ± 1.38^{a}
	Pro B2	42.66 ± 0.22	27.09 ± 0.76^{b}	30.97 ± 0.20^{a}	25.85 ± 1.24	5.73 ± 0.03^{b}	12.53 ± 0.04^{a}
	Phenolic acids						
	Gallic acid	0.04 ± 0.01	0.83 ± 0.02^{b}	1.74 ± 0.03^{a}	1.36 ± 0.02	1.29 ± 0.00^{b}	1.22 ± 0.02^{a}
	Caffeic acid	1.86 ± 0.05	1.26 ± 0.01^{b}	1.08 ± 0.05^{a}	1.83 ± 0.07	1.71 ± 0.01^{b}	1.82 ± 0.01^{a}
	Ferulic acid	3.11 ± 0.01	2.22 ± 0.01^{b}	3.21 ± 0.02^{a}	2.42 ± 0.01	0.92 ± 0.02^{b}	2.89 ± 0.13^{a}
	Stilbenes						
	Resveratrol	0.65 ± 0.03	0.83 ± 0.04^{b}	1.08 ± 0.00^{a}	0.73 ± 0.21	1.58 ± 0.01^{b}	1.60 ± 0.00^{a}
	Total non-Ant	182.14 ± 1.88	103.52 ± 1.64^{b}	121.62 ± 4.88^{a}	162.93 ± 4.83	106.89 ± 2.89^{b}	139.46 ± 4.43^{a}
60°C	*Flavanols*						
	(+)-Cat	80.69 ± 2.80	79.87 ± 2.02^{b}	98.60 ± 0.40^{a}	106.73 ± 1.73	100.36 ± 0.07^{b}	101.91 ± 0.40^{a}
	(-)- Epi	105.35 ± 1.56	98.73 ± 0.01^{b}	113.87 ± 2.87^{a}	101.16 ± 0.88	117.21 ± 0.85^{b}	96.64 ± 0.76^{a}
	(-)- Epig	33.41 ± 1.10	16.77 ± 0.62^{b}	32.81 ± 1.86^{a}	45.87 ± 0.33	32.88 ± 0.60^{b}	42.45 ± 1.73^{a}
	(-)- EpiG	237.82 ± 1.29	177.90 ± 0.63^{b}	187.32 ± 1.54^{a}	265.23 ± 2.38	213.25 ± 2.38^{b}	241.18 ± 1.84^{a}
	Pro B1	39.83 ± 0.70	58.85 ± 2.30^{b}	66.51 ± 2.50^{a}	53.11 ± 1.54	45.75 ± 0.64^{b}	72.44 ± 0.07^{a}
	Pro B2	115.75 ± 2.22	90.10 ± 0.37^{b}	105.98 ± 2.93^{a}	145.91 ± 2.08	120.63 ± 0.01^{b}	138.09 ± 1.38^{a}
	Phenolic acids						
	Gallic acid	n.d	2.63 ± 0.01^{b}	3.66 ± 0.00^{a}	0.62 ± 0.02	0.18 ± 0.00^{b}	0.85 ± 0.09^{a}
	Caffeic acid	2.28 ± 0.22	6.75 ± 0.01^{b}	8.17 ± 0.04^{a}	3.30 ± 0.02	10.40 ± 0.40^{a}	4.17 ± 0.00^{b}
	Ferulic acid	10.93 ± 0.21	6.80 ± 0.15^{b}	7.55 ± 0.08^{a}	20.17 ± 0.86	34.70 ± 0.38^{a}	9.64 ± 1.24^{b}
	Stilbenes						
	Resveratrol	4.82 ± 0.02	3.53 ± 0.00^{b}	6.88 ± 0.07^{a}	3.94 ± 0.84	3.33 ± 0.23^{b}	8.26 ± 0.11^{a}
	Total non-Ant	630.88 ± 10.12	541.93 ± 6.12^{b}	631.35.35 ± 12.29^{a}	746.04 ± 10.68	678.69 ± 5.56^{b}	715.63 ± 7.62^{a}

70°C	*Flavanols*						
	(+)-Cat	155.57 ± 0.28	81.60 ± 0.68^{b}	125.60 ± 0.51^{a}	186.89 ± 0.64	77.55 ± 1.01^{b}	131.03 ± 0.56^{a}
	(-)- Epi	122.17 ± 0.51	103.6 ± 2.93^{a}	117.39 ± 1.58^{b}	153.59 ± 0.43	157.93 ± 1.51^{a}	115.97 ± 0.53^{b}
	(-)- Epig	38.24 ± 0.16	32.63 ± 0.48^{b}	35.17 ± 0.08^{a}	50.12 ± 0.06	41.34 ± 2.81^{b}	47.99 ± 1.49^{a}
	(-)- EpiG	307.65 ± 0.52	245.45 ± 1.56^{b}	295.21 ± 0.82^{a}	402.28 ± 1.08	398.90 ± 1.61^{a}	401.90 ± 1.80^{a}
	Pro B1	50.40 ± 0.21	66.62 ± 1.00^{b}	77.92 ± 0.39^{a}	99.80 ± 1.99	76.99 ± 0.18^{b}	84.64 ± 0.57^{a}
	Pro B2	174.47 ± 0.03	150.52 ± 0.24^{b}	160.51 ± 0.96^{a}	178.22 ± 1.40	150.65 ± 0.66^{b}	161.92 ± 0.46^{a}
	Phenolic acids						
	Gallic acid	n.d	7.92 ± 0.01^{b}	9.18 ± 0.00^{a}	8.22 ± 0.06	6.19 ± 1.15^{a}	6.20 ± 0.03^{a}
	Caffeic acid	10.57 ± 0.00	9.54 ± 0.16^{b}	10.64 ± 0.17^{a}	4.71 ± 0.16	11.67 ± 0.42^{a}	6.95 ± 0.09^{b}
	Ferulic acid	7.55 ± 0.10	12.97 ± 0.06^{a}	13.01 ± 0.79^{a}	9.70 ± 0.04	21.46 ± 1.38^{a}	11.32 ± 0.45^{b}
	Stilbenes						
	Resveratrol	3.84 ± 0.00	3.29 ± 0.04^{b}	5.96 ± 0.02^{a}	3.96 ± 0.08	3.25 ± 0.28^{b}	8.03 ± 0.76^{a}
	Total non-Ant	870.46 ± 1.81	714.14 ± 7.16^{b}	850.59 ± 5.32^{a}	1097.49 ± 5.88	945.93 ± 11.01^{a}	972.95 ± 6.74^{a}
80°C	*Flavanols*	-	-	-			
	(+)-Cat	-	-	-	167.20 ± 0.53	124.94 ± 0.10^{b}	133.75 ± 1.63^{a}
	(-)- Epi	-	-	-	157.77 ± 0.37	146.16 ± 0.89^{a}	82.56 ± 0.31^{b}
	(-)- Epig	-	-	-	30.46 ± 0.64	20.99 ± 1.28^{b}	27.42 ± 0.52^{a}
	(-)- EpiG	-	-	-	308.67 ± 2.04	236.38 ± 1.23^{b}	289.82 ± 0.50^{a}
	Pro B1	-	-	-	68.49 ± 0.27	51.78 ± 1.39^{b}	64.10 ± 1.30^{a}
	Pro B2	-	-	-	290.92 ± 0.87	141.19 ± 0.23^{b}	257.14 ± 0.70^{a}
	Phenolic acids						
	Gallic acid	-	-	-	27.10 ± 0.08	14.54 ± 0.23^{b}	15.71 ± 0.02^{a}
	Caffeic acid	-	-	-	12.13 ± 0.11	18.78 ± 0.09^{a}	9.46 ± 0.38^{b}
	Ferulic acid	-	-	-	13.93 ± 0.28	17.13 ± 0.21^{a}	9.85 ± 0.01^{b}
	Stilbenes						
	Resveratrol	-	-	-	1.26 ± 0.04	1.40 ± 0.00^{b}	1.96 ± 0.17^{a}
	Total non-Ant	-	-	-	1077.93 ± 5.15	773.29 ± 5.65^{b}	891.77 ± 5.54^{a}

Mean (n=3) ± SD. For each yeast strain, different letters in the same row indicate significant difference at $p < 0.05$. Cat, catechin; Epi, epicatechin; Epig, epicatechin gallate; EpiG, epigallocatechin; Pro B1, procyanidin B1; Pro B2, procyanidin B2; Ant, anthocyanins

III.4. Effect of grape varieties

The results showed that larger differences were found between wines of two grape varieties. This could indicate that the contribution of the yeast strain to phenolic compound profile could be overwhelmed by the characteristics of the grape varieties. Trying to assess if the wines from both varieties from two distinct regions could be differentiated based on the type of yeasts used, a discriminant analyses was conducted. First, when discriminant analyses were applied on Syrah and Cabernet Sauvignon Saint Thomas (using the 19 variables of the wines detected after alcoholic fermentation), three discriminant functions were obtained. These discriminant functions allowed us to correctly classify 100% of the studied wines (Figure III.1 and Table III.10). Function 1 discriminates wine samples according to yeast strains (wines fermented with Y strain clearly distinguished from those fermented by X), the variables with the highest discriminant power was delphinidin followed by gallic acid, procyanidin B1 and ferulic acid. Morata at al. (2003) also found that glycosylated delphinidin was the anthocyanin most affected by the yeast strain. Function 2 discriminates samples according to grape varieties (Syrah and Cabernet Sauvignon), the variables with the highest discriminant power being total tannins followed by delphinidin, epigallocatechin, ABTS and Resveratrol. At the end, discriminant analyses conducted on the 19 variables of Syrah and Cabernet Sauvignon Florentine showed that wines are mainly separated according to the grape varieties (Figure III.2) and the variable with the highest discriminant power was procyanidin B2 followed by peonidin, delphinidin, caffeic acid and total polyphenol (Table III.11).

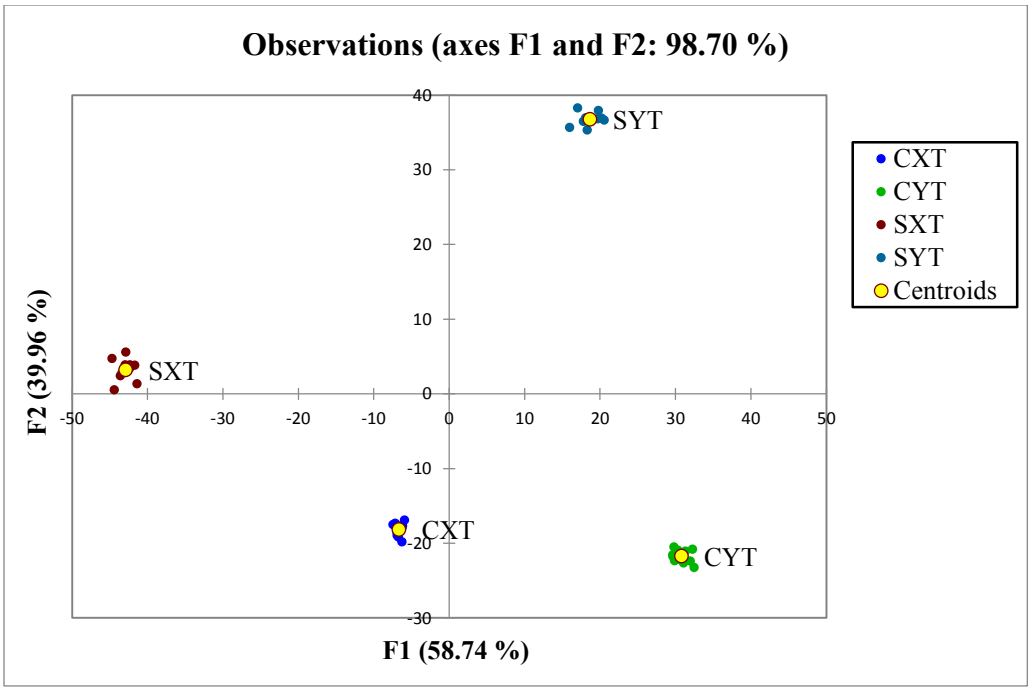

Figure III.1: Distribution of the Thomas wines in the coordinate system defined by the discriminant function to differentiate among wines fermented with two different yeast strains (CXT, Cabernet Sauvignon Saint Thomas wines fermented by X strain; CYT, Cabernet Sauvignon Saint Thomas wines fermented by Y strain; SXT, Syrah Saint Thomas wines fermented by X strain; SYT, Syrah Saint Thomas wines fermented by Y strain)

Table III.10: Standardized coefficients for the three discriminant functions

	F1	F2	F3
TA	-20.001	-60.029	23.529
Dp	67.262	40.995	9.643
Cy	8.876	-5.516	1.419
Pn	-10.679	-0.997	1.708
Mv	-0.372	-2.286	-17.656
TPI	-6.606	-13.116	13.452
TP	-4.608	-62.462	-33.796
T	-27.096	51.984	-17.362
ABTS	-3.483	24.417	1.434
G.A	27.026	-17.068	5.108
Pro B1	17.318	14.544	-11.021
EpiG	17.251	33.777	-18.839
cat	-23.384	-38.191	4.579
Pro B2	-19.801	-15.456	7.149
C.A	-9.047	3.148	13.795
Epi	5.231	-6.961	7.101
Epig	-13.825	-7.294	11.008
F.A	16.276	14.564	3.290
Res	5.298	17.368	-0.948

Abbreviations: TA, total anthocyanins; Dp, delphinidin-3-O-glucoside; Cy, cyanidin-3-O-glucoside; Pn, peonidin-3-O-glucoside; Mv, malvidin-3-O-glucoside, TPI, total phenolic index, TP, total phenolic; T. Tannins; G.A, gallic acid; Pro B1, procyanidin B1; EpiG, epigallocatechin; Cat, catechin; Pro B2, procyanidin B2; C.A, caffeic acid, Epi, epicatechin; Epig, epicatechin gallate; F.A, ferulic acid; Res, resveratrol

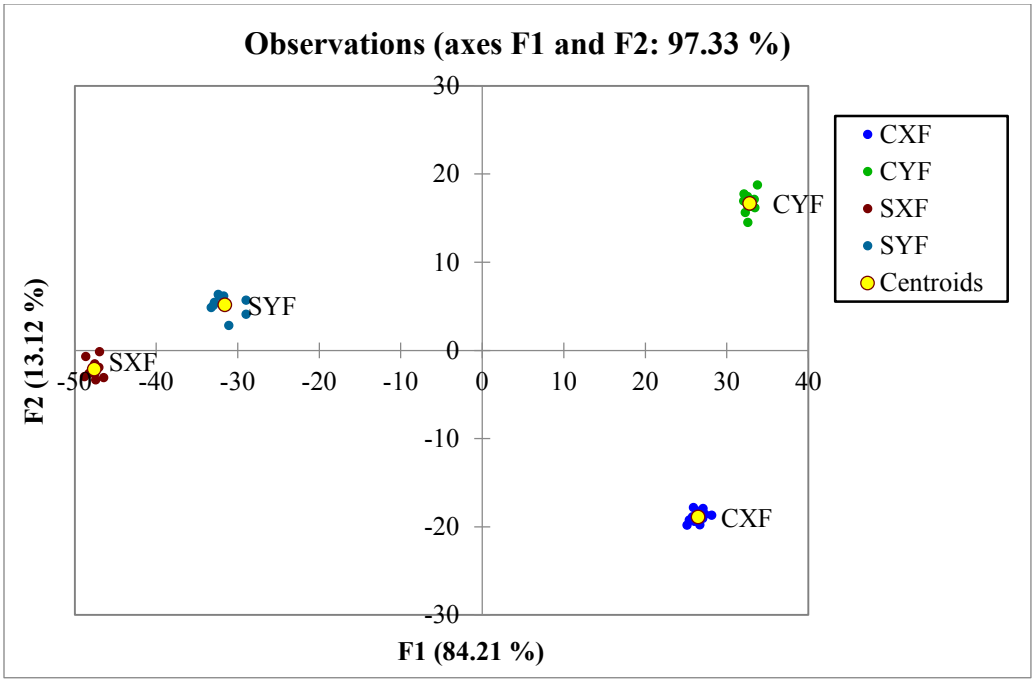

Figure III.2: Distribution of the Florentine wines in the coordinate system defined by the discriminant function to differentiate among wines fermented with two different yeast strains (CXF, Cabernet Sauvignon Florentine wines fermented by X strain; CYF, Cabernet Sauvignon Florentine wines fermented by Y strain; SXF, Syrah Florentine wines fermented by X strain; SYF, Syrah Florentine wines fermented by Y strain)

Table III.11: Standardized coefficients for the three discriminant functions

	F1	F2	F3
TA	-23.681	-2.733	-16.920
Dp	27.974	5.615	11.732
Cy	-16.183	-6.822	-2.442
Pn	39.902	18.438	-17.370
Mv	2.921	5.830	1.403
TPI	-21.459	72.358	-0.886
TP	18.498	-51.003	-29.351
T	-27.012	-81.695	25.116
ABTS	-16.450	-0.644	6.118
G.A	8.295	1.287	-23.086
Pro B1	-13.906	5.043	10.669
EpiG	12.520	28.941	3.572
cat	-9.086	3.114	8.806
Pro B2	46.274	-2.370	-11.947
C.A	21.529	17.342	6.952
Epi	2.839	4.691	-15.544
Epig	5.922	9.805	1.433
F.A	-13.028	-4.605	10.461
Res	-7.859	2.707	7.814

Abbreviations: TA, total anthocyanins; Dp, delphinidin-3-O-glucoside; Cy, cyanidin-3-O-glucoside; Pn, peonidin-3-O-glucoside; Mv, malvidin-3-O-glucoside, TPI, total phenolic index, TP, total phenolic; T. Tannins; G.A, gallic acid; Pro B1, procyanidin B1; EpiG, epigallocatechin; Cat, catechin; Pro B2, procyanidin B2; C.A, caffeic acid, Epi, epicatechin; Epig, epicatechin gallate; F.A, ferulic acid; Res, resveratrol

III.5. Phenolic composition of CS from the two different terroir

In this part, CS wines of the different terroirs will be compared together depending on the yeast strain used. With regards to anthocyanin content, phenolic profile and antioxidant activity (Table III.12), differences between Cabernet Sauvignon from the two different regions were significant. CS-F-60°C wines presented higher values of total anthocyanin. A detectable maximum average drop of almost 17.55%, 38.37%, 23.91% and 17.66% in total anthocyanins were recorded after alcoholic fermentation respectively at temperatures of 10°C, 60°C, 70°C and 80°C (Table III.12) for the two studied regions. X strain lead the lowest total anthocyanin contents. Moreover, CS-F fermented wines premacerated at 70°C showed respectively higher values of total polyphenol index, total polyphenols, tannins and antioxidant activities (83.87; 4390 (mg/l GAE); 4732.76 (mg/l) and 2.50 (mg/ml)). After alcoholic fermentation a significant decrease in the wine phenolic compounds and antioxidant activities was observed. The maximum values of TPI, TP,

T and ABTS were found when X strain was used. Besides, X strain also showed higher polyphenols content than Y strain. CS Saint Thomas and Florentine had in most of the cases the same antioxidant activities which shows that phenolic compounds does not have the same contribution to the antioxidant activities (Rice-Evans et al., 1997). As already seen, Y strain showed higher concentration of anthocyanin while X strain revealed higher content of total non-anthocyanin compound pointing to more hydrophilic parietal constituent and β-glucosidase activity for X strain.

Table III.12: Total anthocyanin, phenolic profile, and antioxidant activity in wines from *Vitis vinifera* L. cv. Cabernet Sauvignon Saint Thomas and Florentine of 2014 vintage, resulting from the alcoholic fermentation of the must premacerated at different temperatures (10°C, 60°C, 70°C and 80°C) with two different yeast strains.

		CS-ST-2014			CS-F-2014		
			Y	X		Y	X
		T0	TF	TF	T0	TF	TF
10°C	TA	78.44 ± 0.49	69.12 ± 0.00^{a}	65.62 ± 2.65^{a}	80.88 ± 1.02	68.02 ± 2.22^{a}	65.70 ± 0.70^{a}
	TPI	15.70 ± 0.00	13.57 ± 0.11^{b}	15.60 ± 0.17^{b}	24.63 ± 1.15	15.53 ± 0.06^{a}	18.61 ± 0.15^{a}
	TP	891.67 ± 2.89	515.00 ± 13.23^{b}	851.67 ± 2.89^{b}	1025.67 ± 6.03	861.67 ± 2.89^{a}	1006.33 ± 5.50^{a}
	T	832.38 ± 3.87	367.40 ± 0.48^{b}	502.55 ± 0.05^{b}	947.40 ± 2.28	489.93 ± 0.03^{a}	917.69 ± 2.68^{a}
	ABTS	0.001 ± 0.00	0.001 ± 0.00	0.001 ± 0.00	0.001 ± 0.00	0.001 ± 0.00	0.001 ± 0.00
60°C	TA	571.57 ± 2.87	346.06 ± 2.87^{b}	340.87 ± 3.03^{b}	574.71 ± 17.54	403.96 ± 1.01^{a}	365.57 ± 0.28^{a}
	TPI	68.71 ± 1.70	56.80 ± 0.18^{b}	65.29 ± 0.27^{a}	71.45 ± 0.45	67.67 ± 0.11^{a}	70.40 ± 0.17^{a}
	TP	3968.33 ± 10.40	2638.33 ± 16.07^{b}	2925.00 ± 5.00^{b}	4093.33 ± 2.89	3353.33 ± 5.77^{a}	3476.67 ± 2.89^{a}
	T	7477.29 ± 1.58	3382.55 ± 0.36^{b}	3955.20 ± 23.24^{b}	10349.91 ± 25.89	3593.69 ± 2.60^{a}	4434.28 ± 11.15^{a}
	ABTS	2.35 ± 0.02	3.25 ± 0.00^{a}	2.90 ± 0.00^{a}	1.73 ± 0.05	3.30 ± 0.00^{a}	3.57 ± 0.00^{b}
70°C	TA	259.21 ± 4.09	239.66 ± 1.80^{a}	235.25 ± 1.52^{a}	298.11 ± 0.53	226.92 ± 11.11^{a}	183.37 ± 2.19^{b}
	TPI	87.45 ± 0.35	77.97 ± 1.08^{b}	80.13 ± 3.26^{b}	90.60 ± 0.17	80.40 ± 0.00^{a}	83.87 ± 0.81^{a}
	TP	4000.67 ± 0.58	3723.33 ± 15.27^{a}	3976.67 ± 7.64^{b}	4750 ± 30	3378.67 ± 105.40^{b}	4290.33 ± 93.05^{a}
	T	10551.27 ± 3.87	4245.05 ± 6.66^{b}	4400.62 ± 136.83^{b}	11734.91 ± 0.98	4656.96 ± 2.70^{a}	4732.76 ± 10.94^{a}
	ABTS	2.00 ± 0.00	2.25 ± 0.05^{b}	2.52 ± 0.13^{a}	3.95 ± 0.17	3.00 ± 0.06^{a}	2.50 ± 0.06^{a}
80°C	TA	61.25 ± 0.00	50.51 ± 1.00^{b}	49.89 ± 1.43^{b}	72.04 ± 2.78	68.75 ± 0.08^{a}	59.96 ± 0.4^{a}
	TPI	85.53 ± 0.12	63.53 ± 0.47^{a}	65.77 ± 0.41^{a}	89.60 ± 0.17	64.07 ± 1.41^{a}	70.30 ± 0.29^{b}
	TP	3215.67 ± 2.89	2978.33 ± 7.64^{b}	3076.74 ± 7.61^{b}	3555 ± 104.49	3042.00 ± 42.64^{a}	3173.33 ± 52.89^{a}
	T	6079.52 ± 19.17	2858.52 ± 14.70^{b}	3159.74 ± 10.61^{b}	8244.83 ± 0.81	2958.99 ± 44.31^{a}	3575.51 ± 87.42^{a}
	ABTS	2.42 ± 0.14	3.81 ± 0.02^{b}	4.01 ± 0.06^{a}	3.10 ± 0.06	4.42 ± 0.06^{a}	4.40 ± 0.17^{a}

Mean (n=3) ± SD. For each yeast strain from different varietal, different letters in the same row indicate significant difference at $p < 0.05$. TA, total anthocyanin; TPI, total phenolic index, TP, total phenolic; T. Tannins.

Table III.13 showed individual monomeric anthocyanin content of wines at the end of alcoholic fermentation. The maximum content of monomeric anthocyanins was found at must macerated at 60°C after 48 hours of maceration, followed by must macerated at 10°C, 70°C and 80°C (Table III.13). Values were 1.8 times higher for Cabernet Sauvignon Florentine (148.75 mg/l) than Cabernet Sauvignon Saint Thomas (80.98 mg/l). After alcoholic fermentation a decrease of the total monomeric anthocyanin was exhibited for the two wine regions fermented by the two yeast strains. CS-F-X fermented wines showed the higher decrease, percentage decrease values were -34.09; -64.04; - 45.47 and -29.40% respectively for the must macerated at temperatures of 10°C, 60°C, 70°C and 80°C. The maximum values of monomeric anthocyanins were found when Y strain was used.

Table III.13: Individual anthocyanin concentration (mg/l) in wines from *Vitis vinifera* L. cv. Cabernet Sauvignon Saint Thomas and Florentine of 2014 vintage resulting from the alcoholic fermentation of the must premacerated at different temperatures (10°C, 60°C, 70°C and 80°C) with two different yeast strains

		CS-ST-2014			CS-F-2014		
			Y	X		Y	X
	Simple glucosides	T0	TF	TF	T0	TF	TF
10°C	Dp-3-glc	3.66 ± 0.01	3.51 ± 0.10^{b}	3.21 ± 0.12^{b}	4.90 ± 0.03	4.60 ± 0.04^{a}	4.58 ± 0.05^{a}
	Cy-3-glc	1.87 ± 0.02	1.79 ± 0.03^{a}	1.13 ± 0.02^{a}	n.d	n.d	n.d
	Pn-3-glc	1.29 ± 0.00	n.d	0.73 ± 0.00^{b}	1.98 ± 0.07	1.06 ± 0.01^{a}	0.92 ± 0.02^{a}
	Mv-3-glc	14.84 ± 0.07	15.65 ± 0.05^{b}	11.40 ± 0.49^{a}	18.81 ± 0.06	18.10 ± 0.70^{a}	11.43 ± 0.32^{a}
	ΣAnt-glc	21.66 ± 0.10	20.95 ± 0.18^{b}	16.47 ± 0.63^{a}	25.69 ± 0.16	23.76 ± 0.75^{a}	16.93 ± 0.39^{a}
60°C	Dp-3-glc	5.64 ± 0.01	5.74 ± 0.01^{b}	5.47 ± 0.18^{b}	6.97 ± 0.25	6.92 ± 0.28^{a}	6.90 ± 0.06^{a}
	Cy-3-glc	n.d	n.d	n.d	3.68 ± 0.17	3.15 ± 0.01^{a}	2.95 ± 0.04^{a}
	Pn-3-glc	3.04 ± 0.00	0.95 ± 0.01^{b}	0.97 ± 0.00^{b}	9.58 ± 0.08	2.46 ± 0.02^{a}	2.35 ± 0.11^{a}
	Mv-3-glc	72.30 ± 0.18	30.22 ± 0.15^{b}	29.06 ± 0.14^{b}	128.52 ± 0.11	56.72 ± 0.47^{a}	41.29 ± 0.78^{a}
	ΣAnt-glc	80.98 ± 0.19	36.91 ± 0.17^{b}	35.50 ± 0.32^{b}	148.75 ± 0.61	69.25 ± 0.78^{a}	53.49 ± 0.99^{a}
70°C	Dp-3-glc	6.14 ± 0.48	6.05 ± 0.02^{a}	5.34 ± 0.44^{a}	5.59 ± 0.01	5.46 ± 0.41^{b}	5.40 ± 1.53^{a}
	Cy-3-glc	n.d	n.d	n.d	1.43 ± 0.02	n.d	n.d
	Pn-3-glc	1.02 ± 0.03	0.82 ± 0.01^{a}	0.98 ± 0.02^{a}	1.55 ± 0.03	0.81 ± 0.00^{a}	0.82 ± 0.03^{b}
	Mv-3-glc	11.86 ± 0.05	8.34 ± 0.04^{b}	7.47 ± 0.27^{a}	18.17 ± 0.05	9.96 ± 0.26^{a}	8.36 ± 0.52^{a}
	ΣAnt-glc	19.02 ± 0.56	15.21 ± 0.07^{b}	13.79 ± 0.73^{b}	26.74 ± 0.11	16.23± 0.67^{a}	14.58 ± 2.08^{a}
80°C	Dp-3-glc	4.53 ± 0.01	4.40 ± 0.06^{a}	3.82 ± 0.40^{a}	5.77 ± 0.30	4.24 ± 0.11^{a}	3.46 ± 0.11^{a}
	Cy-3-glc	n.d	n.d	n.d	n.d	n.d	n.d
	Pn-3-glc	n.d	n.d	n.d	n.d	n.d	n.d
	Mv-3-glc	2.27 ± 0.00	2.21 ± 0.02^{a}	2.15 ± 0.00^{a}	2.19 ± 0.01	2.25 ± 0.00^{a}	2.16 ± 0.01^{a}
	ΣAnt-glc	6.80 ± 0.01	6.61 ± 0.06^{a}	5.97 ± 0.40^{a}	7.96 ± 0.31	6.49 ± 0.11^{a}	5.62 ± 0.05^{a}

Mean (n=3) ± SD. For each yeast strain from different varietal, different letters in the same row indicate significant difference at $p < 0.05$. Dp, delphinidin; Cy, cyanidin; Pn, peonidin; Mv, malvidin; glc, glucoside; Ant, anthocyanin; n.d, not detected values.

In agreement with the results obtained from total polyphenols (Table III.12), Cabernet Sauvignon Florentine must premacerated at 70°C showed the highest content in total non-anthocyanins phenolic compounds with a concentration of 1093.53 mg/l (Table III.14). After alcoholic fermentation, a maximum drop of almost 58.91% and 33.71% in total non-anthocyanins phenolic compounds content was showed respectively for Cabernet Sauvignon Saint Thomas wines fermented by Y and X strains at 10°C (Table III.14). The maximum concentration was observed when X strain was used. With regards to flavanol profiles, as detected by HPLC (Table III.14), Epigallocatechin was the most abundant flavanol in Cabernet sauvignon from the two distinct regions. With the exception of epicatechin (from CS-F-Y fermented wines premacerated at temperatures of 60°C and 70°C) and Pro B1 **(**from CS-F-X fermented wines premacerated at 60°C) which showed an increase of 15.86 and 2.82% on the epicatechin concentrations and 36.39% pro B1 concentration. All individual flavanols compounds showed a decrease in their concentrations after alcoholic fermentation. In addition, the sum of flavanols decreased by an average factor of 1.64 and 1.21 respectively for CS-ST Y and X fermented wines and by an average of 1.33 and 1.13 respectively for CS-F Y and X fermented wines. Thus the highest level of flavanols was observed for CS-F and when X yeast strain was used. As for hydroxybenzoic acids, a decrease in gallic acid concentrations was observed after alcoholic fermentation for both yeast strains and at different must temperatures. The maximum drop was shown (Table III.14) at CS-F-Y premacerated at 80°C (- 46.34%). Regarding hydroxycinnamic acids, an increase in the concentration of caffeic and ferulic acid was observed after alcoholic fermentation. CS-F-Y fermented wines at temperature of 60°C, 70°C and 80°C produced more caffeic and ferulic acid compared to CS-ST-Y fermented wines by the same yeast strain. Concentration of caffeic and ferulic acid average 4.17; 2.20 and 1.97 times higher for CS-F-Y fermented wines compared to CS-ST-Y fermented wines respectively at temperature of 60°C, 70°C and 80°C. This increase in both caffeic and ferulic acids is probably due to the hydrolysis of both caffeic and ferulic tartaric acid esters (Ginjom et al., 2011) found in grapes during winemaking process which produces an increase in free caffeic and ferulic acid in finished wines. Finally, concerning resveratrol**,** as seen in Table III.14, the behavior of the two yeast strains varies depending on the temperature of the must and on the origin of the grapes (two different terroirs). At temperature of 10°C, fermented wines by strain X for the two different grapes regions showed the same value (1.60 mg/l) while fermented wines by strain Y at same temperature showed a concentration 7.52 times higher for

CS-F (1.58 mg/l) compared to CS-ST (0.21 mg/l). Whereas, Cabernet Sauvignon Saint Thomas and Florentine wines fermented by X strain and premacerated at 60°C and 70°C showed a significant increase in the content of resveratrol. This augmentation was nearly 3.7 and 2 times higher respectively for CS-ST and CS-F (relative to the initial value), while Y fermented wines at the same temperatures showed a slight decrease. On the contrary, at temperature of 80°C, CS-F showed the highest content of resveratrols (1.96 mg/l).

Table III.14: Individual non-anthocyanin phenolic compounds (mg/l) in wines from *Vitis vinifera* cv. Cabernet Sauvignon Saint Thomas and Florentine of 2014 vintage resulting from the alcoholic fermentation of the must premacerated at different temperatures (10°C, 60°C, 70°C and 80°C) with two different yeast strains

		CS-ST-2014			CS-F-2014		
			Y	X		Y	X
		T0	TF	TF	T0	TF	TF
10°C	*Flavanols*						
	(+)-Cat	29.00 ± 0.67	3.67 ± 0.07^{b}	6.18 ± 0.09^{b}	25.53 ± 0.25	7.59 ± 0.00^{a}	24.38 ± 1.08^{a}
	(-)- Epi	20.13 ± 0.01	17.43 ± 0.42^{a}	19.57 ± 0.21^{b}	27.54 ± 0.01	17.76 ± 0.34^{a}	21.13 ± 1.46^{a}
	(-)- Epig	6.04 ± 0.00	3.71 ± 0.04^{b}	4.17 ± 0.01^{b}	7.69 ± 0.24	4.63 ± 0.03^{a}	6.19 ± 0.22^{a}
	(-)- EpiG	56.23 ± 0.57	13.64 ± 0.30^{b}	43.17 ± 0.42^{b}	55.42 ± 2.74	46.77 ± 2.44^{a}	54.19 ± 0.09^{a}
	Pro B1	13.30 ± 0.43	7.55 ± 0.02^{b}	9.27 ± 0.12^{b}	14.56 ± 0.06	8.91 ± 0.01^{a}	13.51 ± 1.38^{a}
	Pro B2	13.85 ± 0.10	9.82 ± 0.23^{a}	7.94 ± 0.02^{b}	25.85 ± 1.24	5.73 ± 0.03^{b}	12.53 ± 0.04^{a}
	Σflavanols	138.55 ±1.78	55.82± 1.08	90.30 ± 0.87	156.59 ± 4.54	91.39 ± 2.85	131.93 ± 4.27
	Phenolic acids						
	Gallic acid	0.17 ± 0.00	0.20 ± 0.00^{b}	0.17 ± 0.00^{b}	1.36 ± 0.02	1.29 ± 0.00^{a}	1.22± 0.02^{a}
	Caffeic acid	2.00 ± 0.00	1.26 ± 0.02^{b}	1.54 ± 0.02^{b}	1.83 ± 0.07	1.71 ± 0.01^{a}	1.82 ± 0.01^{a}
	Ferulic acid	2.47 ± 0.13	2.06 ± 0.08^{a}	2.45 ± 0.03^{b}	2.42 ± 0.01	0.92 ± 0.02^{b}	2.89 ± 0.13^{a}
	Stilbenes						
	Resveratrol	1.74 ± 0.01	0.21 ± 0.00^{b}	1.61 ± 0.00^{a}	0.73 ± 0.21	1.58 ± 0.01^{a}	1.60 ± 0.00^{a}
	Total non-Ant	144.93 ± 1.92	59.55 ± 1.18^{b}	96.07 ± 0.92^{b}	162.93 ± 4.83	96.89 ± 2.89^{a}	139.46 ± 4.43^{a}
60°C	*Flavanols*						
	(+)- Cat	143.30 ± 1.67	29.17 ± 0.66^{b}	100.84 ± 0.11^{a}	106.73 ± 1.73	100.36 ± 0.07^{a}	101.91 ± 0.40^{a}
	(-)- Epi	92.86 ± 0.88	73.35 ± 0.19b	92.55 ± 0.16^{b}	101.16 ± 0.88	117.21 ± 0.85^{a}	96.64 ± 0.76^{a}
	(-)- Epig	48.75 ± 0.17	35.10 ± 0.36^{a}	45.10 ± 1.27^{a}	45.87 ± 0.33	32.88 ± 0.60^{b}	42.45 ± 1.73^{a}
	(-)- EpiG	259.10 ± 6.22	144.11 ± 1.20^{b}	239.87 ± 7.12^{a}	265.23 ± 2.38	213.25 ± 2.38^{a}	241.18 ± 1.84^{a}
	Pro B1	69.05 ± 1.04	36.29 ± 0.23^{b}	50.41 ± 2.50^{b}	53.11 ± 1.54	45.75 ± 0.64^{a}	72.44 ± 0.07^{a}
	Pro B2	84.94 ± 1.09	72.46 ± 0.35^{b}	85.25 ± 0.02^{b}	145.91 ± 2.08	120.63 ± 0.01^{a}	138.09 ± 1.38^{a}
	Σflavanols	698.00 ± 11.07	390.48 ± 2.99^{b}	614.02 ± 11.18^{b}	718.01 ± 8.94	630.08 ± 4.55^{a}	692.71 ± 6.18^{a}
	Phenolic acids						
	Gallic acid	1.71 ± 0.00	1.00 ± 0.00^{a}	1.44 ± 0.05^{a}	0.62 ± 0.02	0.18 ± 0.00^{b}	0.85 ± 0.09^{b}
	Caffeic acid	3.61 ± 0.17	2.53 ± 0.01^{b}	3.77 ± 0.02^{b}	3.30 ± 0.02	10.40 ± 0.40^{a}	4.17 ± 0.00^{a}
	Ferulic acid	7.18 ± 0.10	8.15 ± 0.03^{b}	10.40 ± 0.04^{a}	20.17 ± 0.86	34.70 ± 0.38^{a}	9.64 ± 1.24^{a}
	Stilbenes						
	Resveratrol	2.60 ± 0.04	2.42 ± 0.01^{b}	9.10 ± 0.03^{a}	3.94 ± 0.84	3.33 ± 0.23^{a}	8.26 ± 0.11^{b}
	Total non-Ant	713.10 ± 11.38	404.58 ± 3.04^{b}	638.73 ± 11.32^{b}	746.04 ± 10.68	678.69 ± 5.56^{a}	715.63 ± 7.62^{a}

70°C	*Flavanols*						
	(+)- Cat	119.41 ± 2.14	96.62 ± 1.56^{a}	116.24 ± 2.00^{b}	186.89 ± 0.64	77.55 ± 1.01^{b}	131.03 ± 0.56^{a}
	(-)- Epi	156.80 ± 2.69	109.32 ± 3.30^{b}	131.26 ± 2.27^{a}	153.59 ± 0.43	157.93 ± 1.51^{a}	115.97 ± 0.53^{b}
	(-)- Epig	58.31 ± 0.23	48.39 ± 0.32^{a}	56.08 ± 3.45^{a}	50.12 ± 0.06	41.34 ± 2.81^{b}	47.99 ± 1.49^{b}
	(-)- EpiG	404.84 ± 1.48	324.43 ± 3.25^{b}	340.85 ± 0.50^{b}	402.28 ± 1.08	398.90 ± 1.61^{a}	401.90 ± 1.80^{a}
	Pro B1	90.89 ± 0.52	65.75 ± 0.71^{b}	75.85 ± 1.17^{b}	99.80 ± 1.99	76.99 ± 0.18^{a}	84.64 ± 0.57^{a}
	Pro B2	169.30 ± 0.40	169.98 ± 0.57^{a}	152.67 ± 0.24^{a}	178.22 ± 1.40	150.65 ± 0.66^{b}	161.92 ± 0.46^{a}
	Σflavanols	999.55 ± 7.46	814.49 ± 9.70^{b}	872.95 ± 9.63^{b}	1070.90 ± 5.60	903.36 ± 7.78^{a}	943.45 ± 5.41^{a}
	Phenolic acids						
	Gallic acid	5.60 ± 0.32	4.22 ± 0.08^{b}	5.15 ± 0.13^{b}	8.22± 0.06	6.19 ± 1.15^{a}	6.20 ± 0.03^{a}
	Caffeic acid	7.04 ± 0.02	5.93 ± 0.04^{b}	6.26 ± 0.05^{b}	4.71 ± 0.16	11.67 ± 0.42^{a}	6.95 ± 0.09^{a}
	Ferulic acid	8.88 ± 0.88	8.85 ± 0.91^{b}	9.35 ± 0.62^{b}	9.70 ± 0.04	21.46 ± 1.38^{a}	11.32 ± 0.45^{a}
	Stilbenes						
	Resveratrol	2.31 ± 0.08	2.10 ± 0.01^{b}	9.21 ± 0.26^{a}	3.96 ± 0.08	3.25 ± 0.28^{a}	8.03 ± 0.76^{a}
	Total non-Ant	1023.38 ± 8.87	835.59 ± 10.75^{b}	902.92 ± 10.69^{b}	1093.53 ± 5.88	945.93 ± 11.01^{a}	972.95 ± 6.74^{a}
80°C	*Flavanols*						
	(+)- Cat	134.53 ± 0.48	126.44 ± 0.23^{a}	128.00 ± 0.52^{b}	167.20 ± 0.53	124.94 ± 0.10^{b}	133.75 ± 1.63^{a}
	(-)- Epi	116.02 ± 0.78	88.90 ± 0.90^{b}	98.73 ± 0.71^{a}	157.77 ± 0.37	146.16 ± 0.89^{a}	82.56 ± 0.31^{b}
	(-)- Epig	45.17 ± 0.88	35.12 ± 1.07^{a}	40.70 ± 0.62^{a}	30.46 ± 0.64	20.99 ± 1.28^{b}	27.42 ± 0.52^{b}
	(-)- EpiG	312.82 ± 0.89	275.64 ± 1.96^{a}	306.59 ± 0.16^{a}	308.67 ± 2.04	236.38 ± 1.23^{b}	289.82 ± 0.50^{b}
	Pro B1	84.35 ± 1.76	62.85 ± 0.45^{a}	68.15 ± 0.09^{a}	68.49 ± 0.27	51.78 ± 1.39^{b}	64.10 ± 1.30^{b}
	Pro B2	138.08 ± 0.05	130.09 ± 5.84^{b}	115.11 ± 1.29^{b}	290.92 ± 0.87	141.19 ± 0.23^{a}	257.14 ± 0.70^{a}
	Σflavanols	830.97 ± 4.84	719.04 ± 10.45	757.28 ± 3.39	1023.51 ± 4.72	721.44 ± 5.12	854.79 ± 4.96
	Phenolic acids						
	Gallic acid	18.22 ± 0.49	14.53 ± 0.15^{a}	15.75 ± 0.28^{a}	27.10 ± 0.08	14.54 ± 0.23^{a}	15.71 ± 0.02^{a}
	Caffeic acid	9.17 ± 0.02	7.84 ± 0.25^{b}	8.73 ± 0.21^{b}	12.13 ± 0.11	18.78 ± 0.09^{a}	9.46 ± 0.38^{a}
	Ferulic acid	11.59 ± 0.18	11.02 ± 0.02^{b}	11.07 ± 0.48^{a}	13.93 ± 0.28	17.13 ± 0.21^{a}	9.85 ± 0.01^{b}
	Stilbenes						
	Resveratrol	1.38 ± 0.01	1.33 ± 0.01^{b}	1.63 ± 0.02^{a}	1.26 ± 0.04	1.40 ± 0.00^{a}	1.96 ± 0.17^{a}
	Total non-Ant	871.33 ± 5.43	753.76 ± 10.88^{b}	794.46 ± 5.38^{b}	1077.93 ± 5.15	773.29 ± 5.65^{a}	891.77 ± 5.54^{a}

Mean (n=3) ± SD. For each yeast strain from different varietal, different letters in the same row indicate significant difference at $p < 0.05$. Cat, catechin; Epi, epicatechin; Epig, epicatechin gallate; EpiG, epigallocatechin; Pro B1, procyanidin B1; Pro B2, procyanidin B2; Ant, anthocyanins

III.6. Terroir Effects

In order to assess if the differences were due to the terroir effect or and to the yeast strains, a discriminant analyses was conducted.

When discriminant analyses were applied on Cabernet Sauvignon Saint Thomas and Florentine (using the 19 variables of the wines detected after alcoholic fermentation), three discriminant functions were obtained. These discriminant functions allowed us to correctly classify 100% of the studied wines (Table III.15). The differences in wine samples were mostly due to the yeast strain effects (Figure III.3). Function 1 discriminates wine samples according to yeast strains (wines fermented with Y clearly distinguished from those fermented by X), the variables with the highest discriminant power was Dp followed by TPI, pro B1, pro B2 and F.A. these results are in accordance with Bartowsky et al., 2004 who stated that the yeast effects were maintained even when using grapes from the same variety but from different sources.

Although the differences in wine samples were due to both terroir and yeast strains effects, but the effect of the latter was the most important (Figure III.3)

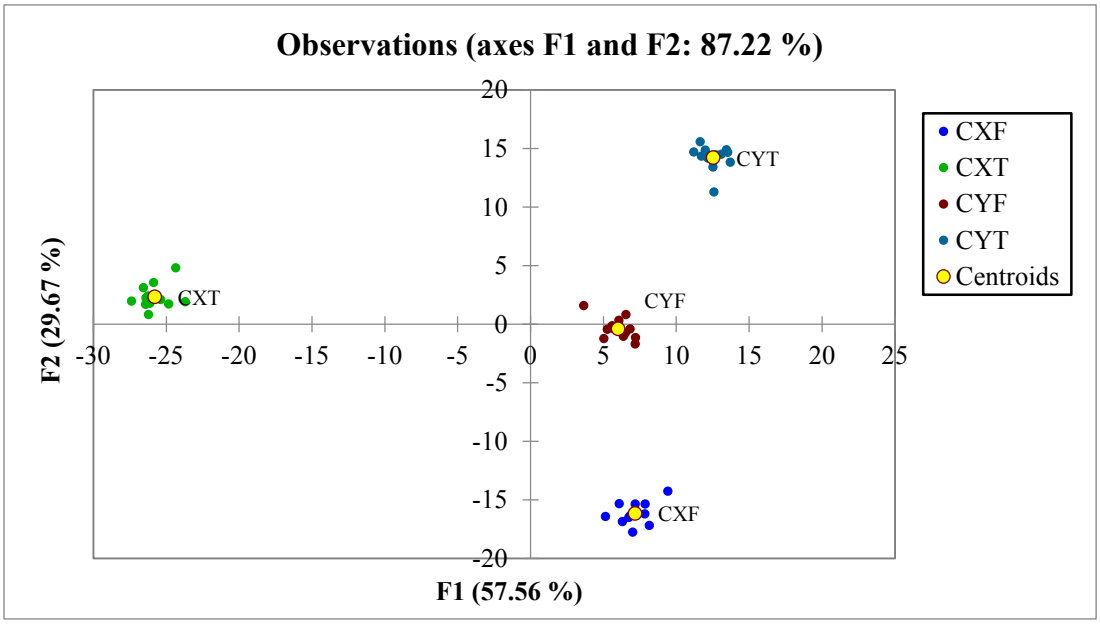

Figure III.3: Distribution of the CS wines in the coordinate system defined by the discriminant function to differentiate among wines fermented with two different yeast strains (CXF, Cabernet Sauvignon Florentine wines fermented by X strain; CXT, Cabernet Sauvignon Saint Thomas wines fermented by X strain; CYF, Cabernet Sauvignon Florentine wines fermented by Y strain; CYT, Cabernet Sauvignon Saint Thomas wines fermented by Y strain

Table III.15: Standardized coefficients for the three discriminant functions

	F1	F2	F3
TA	-9.109	27.549	2.075
Dp	47.578	3.155	4.764
Cy	2.285	4.388	-5.012
Pn	-26.532	-6.927	4.083
Mv	-1.877	-2.160	4.197
TPI	25.884	47.955	24.304
TP	3.013	-11.133	-15.643
T	-34.326	-56.201	-50.534
ABTS	3.520	-1.512	7.760
G.A	2.354	13.685	-8.790
Pro B1	20.780	-24.299	11.370
EpiG	-7.475	21.147	25.761
cat	-20.407	-10.004	-4.247
Pro B2	13.533	14.856	-4.311
C.A	-6.232	-2.131	1.626
Epi	-22.246	2.026	1.022
Epig	-12.804	0.243	2.427
F.A	12.982	-5.619	0.802
Res	6.355	2.109	1.100

TA, total anthocyanin; Dp, delphinidin-3-O-glucoside; Cy, cyanidin-3-O-glucoside; Pn, peonidin-3-O-glucoside; Mv, malvidin-3-O-glucoside, TPI, total phenolic index, TP, total phenolics; T. Tannins; G.A, gallic acid; Pro B1, procyanidin B1; EpiG, epigallocatechin; Cat, catechin; Pro B2, procyanidin B2; C.A, caffeic acid, Epi, epicatechin; Epig, epicatechin gallate; F.A, ferulic acid; Res, resveratrol

III.7. Effect of maceration enzymes on polyphenol composition of wines after alcoholic fermentation

The total anthocyanin, phenolic profiles and antioxidant activity in wines from Syrah and Cabernet Sauvignon Saint Thomas musts macerated at different temperatures with or without added enzymes and fermented by two yeast strains, using spectrophotometric methods to evaluate the influence of maceration enzymes were respectively represented in Table III.16 and III.17. Changes in phenolic compounds were observed at different stages: beginning, middle and final steps of alcoholic fermentation. At the beginning of the fermentation (T0), musts macerated at 70°C + enzymes showed the highest total anthocyanin contents with an average value of 355.66 mg/l for the two grape varieties followed by must macerated at 70°C than the control. During alcoholic fermentation a significant loss of total anthocyanin was observed at the middle stage (T1/2) of Syrah and Cabernet Sauvignon musts macerated at 70°C and 70°C + enzymes to reach a max decrease ranging between 49.42% and 71.43% for the two grape varieties fermented by the two yeast strains. At the end of alcoholic fermentation wine samples premacerated at 70°C + enzymes demonstrated the highest anthocyanin contents, values were approximately two times and more than one time higher respectively than Syrah and Cabernet Sauvignon wines premacerated at 70°C. In addition control wines showed increases in the anthocyanin levels at the beginning of fermentation (maceration and fermentation at the same time) and then a progressive decrease was shown ranging from 42.91% to 71.74% for X strain and 36.34% to 61.42% for Y strain for the two grape varieties. Control wines 25°C + enzymes showed the highest anthocyanin concentrations ($[TA]_{TF\text{-}Sy\text{-}X\text{-}25°C+E}$ = 172.04 mg/l; $[TA]_{TF\text{-}Sy\text{-}Y\text{-}25°C+E}$ = 304.71 mg/l; $[TA]_{TF\text{-}CS\text{-}X\text{-}25°C+E}$ = 207.95 mg/l; $[TA]_{TF\text{-}CS\text{-}Y\text{-}25°C+E}$ = 217.00 mg/l). In fact, the decrease in the level of anthocyanin found in all wines after alcoholic fermentation could be due to the fixation of compounds on yeast solid parts and by reactions of degradation or condensation with tannins or other wine components (Auw et al., 1996; Mayen et al., 1994; Chinnici et al., 2009) and the presence of β-glucosidase activity of certain strain of *Saccharomyces cerevisiae* (Morata et al., 2005). The high anthocyanins levels in enzymed wines are due to the pectolytic activity of the enzyme which promotes the liberation of anthocyanins and other phenolic compounds by degrading cell walls. These results are in accordance with those observed by Parley (1997). As total anthocyanin, the highest total polyphenol content was found in wines that were produced under the condition of maceration enzymes (70°C and 25°C + enzymes). Approximately, 66.93

% and 87.26% of the TP content were conserved respectively in Syrah and Cabernet Sauvignon wine samples premacerated at 70°C and 70°C + enzymes after alcoholic fermentation, Moreover, 92.41% of TP were conserved for the control with and without added enzymes from the two grape varieties and the two yeast strains. At the end of fermentation, maximum TPI and tannins was obtained at the same time as maximum polyphenol concentration, values were 80.93 and 4241.67 mg/l respectively for TPI and Tannins (CS-70°C + enzymes Y strain). After alcoholic fermentation, X and Y fermented wines premacerated at 70°C and 70°C + enzymes showed a decrease in antioxidant activity (IC50 from 2.20 to 4.60 mg/ml) while control wines (25°C; 25°C + enzymes) showed an increase (IC50 from 0.00 to 2.83 mg/ml). Wine samples with added enzymes showed the high antioxidant activities.

Table III.16: Total anthocyanin, Phenolic profiles and antioxidant activity in wines from *Vitis vinifera* L. cv. Syrah Saint Thomas of 2015 vintage, at the beginning (T0), middle (T1/2) and final stages of fermentation (TF) of the must macerated at different temperatures with or without added enzymes (70°C, 70°C + enzyme, 25°C and 25°C + enzymes) and fermented with two different yeast strains (X and Y)

				Sy-ST-2015															
				X								Y							
	T0	T0	T0	T1/2	T1/2	T1/2	T1/2	TF	TF	TF	TF	T1/2	T1/2	T1/2	T1/2	TF	TF	TF	TF
	Control	70°C	70°C + Enz	25°C	25°C + Enz	70°C	70°C + Enz	25°C	25°C + Enz	70°C	70°C + Enz	25°C	25°C + Enz	70°C	70°C + Enz	25°C	25°C + Enz	70°C	70°C + Enz
TA	95.08 ± 0.5	304.25 ± 17.28^{b}	356.42 ± 2.81^{a}	499.83 ± 5.27^{b}	523.62 ± 2.31^{a}	372.96 ± 16.65^{b}	384.21 ± 13.39^{a}	141.21 ± 1.01^{b}	172.04 ± 3.07^{a}	89.83 ± 4.97^{b}	177.62 ± 0.50^{a}	551.25 ± 13.47^{b}	593.83 ± 20.43^{a}	396.96 ± 8.13^{b}	428.04 ± 6.56^{a}	247.02 ± 11.23^{b}	304.71 ± 4.40^{a}	86.92 ± 5.05^{b}	180.25 ± 6.94^{a}
TPI	25.21 ± 0.55	75.90 ± 0.52^{b}	96.00 ± 0.50^{a}	57.50 ± 0.21^{b}	63.10 ± 0.40^{a}	68.23 ± 0.95^{b}	93.83 ± 0.85^{a}	49.46 ± 0.10^{b}	50.36 ± 0.15^{a}	63.70 ± 0.34^{b}	80.5 ± 0.10^{a}	56.53 ± 0.25^{b}	63.06 ± 0.94^{a}	74.50 ± 1.38^{b}	95.07 ± 0.06^{a}	53.93 ± 0.30^{b}	61.30 ± 0.32^{a}	72.4 ± 0.43^{b}	77.37 ± 0.75^{a}
TP	976.67 ± 11.54	4150.67 ± 5.41^{b}	4271.67 ± 2.69^{a}	2515.67 ± 120.34^{b}	2560.00 ± 10.00^{a}	3030.33 ± 130.41^{b}	3900.67 ± 170.61^{a}	2275.33± 8.66^{b}	2320.12 ± 13.22^{a}	2520.33 ± 59.08^{b}	3060.00 ± 65.57^{a}	2445.67 ± 17.55^{b}	2640.33 ± 2.88^{a}	3850.00 ± 164.62^{b}	4330.67 ± 83.11^{a}	2203.33 ± 46.18^{b}	2590.33 ± 2.88^{a}	2361.67 ± 20.20^{b}	3353.00 ± 48.94^{a}
T	1056.71 ± 11.16	7000.16 ± 257.41^{b}	8492.31 ± 238.57^{a}	1449.56 ± 0.00^{b}	1759.04 ± 0.00^{a}	6385.34 ± 82.21^{b}	8067.05 ± 44.76^{a}	1050.26 ± 11.16^{b}	1150.36 ± 11.15^{a}	4261.61 ± 88.58^{b}	5408.52 ± 60.95^{a}	1829.91 ± 80.47^{b}	2029.65 ± 11.16^{a}	6572.20 ± 20.41^{b}	7899.53 ± 42.92^{a}	1204.90 ± 19.33^{b}	1262.89 ± 11.16^{a}	4326.04 ± 45.08^{b}	5576.05 ± 30.85^{a}
ABTS	9.00 ± 0.05	2.60 ± 0.09^{a}	2.20 ± 0.06^{b}	4.00 ± 0.30^{a}	3.50 ± 0.00^{b}	2.35 ± 0.04^{a}	1.87 ± 0.01^{b}	3.8 ± 0.10^{a}	3.40 ± 0.17^{b}	4.06 ± 0.03^{a}	3.47 ± 0.05^{b}	4.30 ± 0.23^{a}	3.30 ± 0.00^{b}	2.65 ± 0.08^{a}	2.20 ± 0.01^{b}	4.00 ± 0.11^{a}	3.23 ± 0.29^{b}	4.60 ± 0.17^{a}	3.70 ± 0.03^{b}

Mean (n=3) ± SD. For each yeast strain from the same maceration temperature and stage of fermentation with or without added enzymes, different letters in the same row indicate significant difference at $p < 0.05$. TA, total anthocyanin; TPI, total polyphenol index; TP, total polyphenol and T, tannins

Table III.17: Total anthocyanin, Phenolic profiles and antioxidant activity in wines from *Vitis vinifera* L. cv. Cabernet Sauvignon Saint Thomas of 2015 vintage, at the beginning (T0), middle (T1/2) and final stages of fermentation (TF) of the must macerated at different temperatures with or without added enzymes (70°C, 70°C + enzyme, 25°C and 25°C + enzymes) and fermented with two different yeast strains (X and Y)

				CS-ST-2015															
				X								Y							
	T0	T0	T0	T1/2	T1/2	T1/2	T1/2	TF	TF	TF	TF	T1/2	T1/2	T1/2	T1/2	TF	TF	TF	TF
	Control	70°C	70°C + Enz	25°C	25°C + Enz	70°C	70°C + Enz	25°C	25°C + Enz	70°C	70°C + Enz	25°C	25°C + Enz	70°C	70°C + Enz	25°C	25°C + Enz	70°C	70°C + Enz
TA	5.25 ± 0.00	290.87 ± 2.62^{b}	354.91 ± 5.82^{a}	350.00 ± 0.87^{b}	364.29 ± 9.27^{a}	170.67 ± 6.68^{b}	201.54 ± 8.76^{a}	145.25 ± 11.37^{b}	207.95 ± 5.65^{a}	124.00 ± 3.15^{b}	136.96 ± 3.15^{a}	294.58 ± 12.81^{b}	356.71 ± 4.97^{a}	200.08 ± 10.69^{b}	230.37 ± 9.46^{a}	187.54 ± 0.50^{b}	217.00 ± 3.31^{a}	123.08 ± 4.82^{b}	137.62 ± 3.81^{a}
TPI	17.17 ± 0.58	73.82 ± 0.63^{b}	86.73 ± 0.58^{a}	54.97 ± 0.05^{b}	58.33 ± 0.41^{a}	64.63 ± 0.63^{b}	82.70 ± 0.52^{a}	49.00 ± 0.11^{b}	51.60 ± 0.30^{a}	59.57 ± 0.30^{b}	79.17 ± 0.51^{a}	58.76 ± 0.68^{b}	60.00 ± 0.50^{a}	65.30 ± 0.85^{b}	84.33 ± 0.57^{a}	51.20 ± 0.11^{b}	57.80 ± 0.10^{a}	60.03 ± 0.06^{b}	80.93 ± 0.91^{a}
TP	555.00 ± 35	4095.33 ± 12.58^{b}	4663.33 ± 18.92^{a}	2360.00 ± 36.05^{b}	2501.33 ± 85.19^{a}	3853.33 ± 33.29^{b}	4376.67 ± 3.09^{a}	2045.00 ± 13.22^{b}	2281.67 ± 110.15^{a}	3438.33 ± 7.64^{b}	3956.67 ± 2.89^{a}	2341.67 ± 67.14^{b}	2555.00 ± 75.49^{a}	3908.33 ± 54.84^{b}	4736.67 ± 29.30^{a}	2255.33 ± 5.77^{b}	2450.67 ± 18.46^{a}	3658.33 ± 50.08^{b}	4241.67 ± 5.77^{a}
T	1152.00 ± 0.00	6610.86 ± 6.69^{b}	8750.04 ± 109.91^{a}	1384.49 ± 40.23^{b}	1503.73 ± 48.64^{a}	5259.04 ± 22.38^{b}	7242.31 ± 12.19^{a}	1035.24 ± 2.32^{b}	1225.75 ± 19.33^{a}	4273.21 ± 86.92^{b}	5225.800 ± 33.48^{a}	1587.95 ± 66.96^{b}	1890.48 ± 40.23^{a}	6014.20 ± 32.32^{b}	7873.75 ± 113.26^{a}	1032.96 ± 22.32^{b}	1160.57 ± 55.80^{a}	4247.44 ± 27.89^{b}	5357.50 ± 40.23^{a}
ABTS	0.00 ± 0.00	3.00 ± 0.05^{a}	2.41 ± 0.00^{b}	11.00 ± 0.00^{a}	9.33 ± 0.05^{b}	2.76 ± 0.11^{a}	2.25 ± 0.10^{b}	3.27 ± 0.05^{a}	2.83 ± 0.00^{b}	3.13 ± 0.02	2.45 ± 0.05	12.00 ± 0.05^{a}	10.50 ± 0.00^{b}	2.70 ± 0.00^{a}	2.30 ± 0.12^{a}	2.95 ± 0.13^{a}	2.93 ± 0.10^{a}	3.23 ± 0.11^{b}	2.63 ± 0.05^{a}

Mean (n=3) ± SD. For each yeast strain from the same maceration temperature and stage of fermentation with or without added enzymes, different letters in the same row indicate significant difference at $p < 0.05$. TA, total anthocyanin; TPI, total polyphenol index; TP, total polyphenol and T, tannin

III.7.1. ANTHOCYANIN PROFILE

The evolution of the main individual anthocyanins concentration (mg/l) during alcoholic fermentation of Syrah and Cabernet Sauvignon musts macerated at different temperatures (25°C and 70°C) with or without added enzymes with two different yeast strains (X and Y) was shown in table III.18 and III.19. At the beginning of fermentation (T0), the must macerated at 70°C + enzymes after 24 hours showed the highest content in total monomeric anthocyanins for the two grape varieties (55.11 and 62.76 mg/l, respectively for Syrah and Cabernet Sauvignon Saint Thomas), followed by musts macerated at 70°C than the control (25°C). Malvidin-3-O-glucoside was the major grape and wine anthocyanins, in agreement with the literature for most *Vitis vinifera* L. varieties (Bakker and Timberlake, 1985).

Depending on the winemaking process, different evolution in the concentrations of total anthocyanins during the different stages of alcoholic fermentation was observed (Table III.18 and III.19). Musts from the two grape varieties macerated at temperatures of 70°C and 70°C + enzymes and fermented by the two yeast strains indicated a significant loss of total anthocyanins between 1.90% and 50.42% (middle stage of fermentation) and with an average loss of 86.26% at the end of fermentation. Wines premacerated at 70°C + enzymes showed significantly the highest content (Table III.18 and III.19). Whereas, total monoglucoside anthocyanin content in the control wines (25°C and 25°C + enzymes) increased during the maceration time (T1/2, maceration and fermentation occur in the same time) and decreases gradually till the end of the fermentation process (total anthocyanin loss between 43.72 and 82.23%), for the two grape varieties and yeast strains, in accordance with other results (Koyama et al., 2007; Sacchi et al., 2005). At the end of alcoholic fermentation, control 25°C + enzymes showed higher levels of anthocyanins for X and Y yeast strains and for the two grape varieties. Therefore, as seen previously, the evolution of the total monomeric anthocyanins and the effect of macerating enzymes during alcoholic fermentation showed the same trend as total anthocyanin contents. Decrease in the level of anthocyanin in all wines after alcoholic fermentation can arise from several causes (Auw et al., 1996; Mayen et al., 1994; Morata et al., 2005), as well as the increase of the amount of polyphenols that is favored by the addition of macerating enzymes (Parley, 1997)

Table III.18: Individual anthocyanin concentrations (mg/l) in wines from *Vitis vinifera* L. cv. Syrah Saint Thomas from the 2015 vintage, at the beginning (T0), middle (T1/2) and final stages of fermentation (TF) of the must macerated at different temperatures with or without added enzymes (70°C, 70°C + enzyme, 25°C and 25°C + enzymes) and fermented with two different yeast strains (X and Y)

	Sy-ST-2015																		
	X											Y							
	T0	T0	T0	T1/2	T1/2	T1/2	T1/2	TF	TF	TF	TF	T1/2	T1/2	T1/2	T1/2	TF	TF	TF	TF
	Control	70°C	70°C + Enz	25°C	25°C + Enz	70°C	70°C + Enz	25°C	25°C + Enz	70°C	70°C + Enz	25°C	25°C + Enz	70°C	70°C + Enz	25°C	25°C + Enz	70°C	70°C + Enz
Dp	1.89 ± 0.08	2.20 ± 0.10^{a}	2.40 ± 0.16^{a}	17.41 ± 0.14^{b}	19.71 ± 0.31^{a}	2.10 ± 0.31^{a}	2.34 ± 0.06^{a}	5.61 ± 0.29^{b}	6.68 ± 0.11^{a}	1.94 ± 0.21^{b}	2.22 ± 0.05^{a}	17.65 ± 0.69^{b}	21.31 ± 0.30^{a}	2.16 ± 0.21^{a}	2.36 ± 0.07^{a}	11.18 ± 0.18^{a}	9.60 ± 0.24^{b}	1.95 ± 0.02^{b}	2.30 ± 0.11^{a}
Cy	1.61 ± 0.05	1.30 ± 0.01^{b}	1.45 ± 0.03^{a}	4.50 ± 0.04^{a}	3.70 ± 0.10^{b}	1.16 ± 0.02^{b}	1.31 ± 0.03^{a}	2.29 ± 0.08^{a}	2.46 ± 0.12^{a}	n.d	n.d	3.83 ± 0.08^{b}	4.64 ± 0.14^{a}	1.21 ± 0.03^{b}	1.42 ± 0.01^{a}	2.95 ± 0.04^{b}	3.43 ± 0.14^{a}	n.d	n.d
Pn	4.48 ± 0.07	4.80 ± 0.01^{b}	5.55 ± 0.03^{a}	21.44 ± 0.33^{a}	17.47 ± 0.47^{b}	4.69 ± 0.08^{b}	5.54 ± 0.03^{a}	3.60 ± 0.04^{b}	4.26 ± 0.03^{a}	0.76 ± 0.01^{b}	0.89 ± 0.02^{a}	18.98 ± 0.35^{b}	22.85 ± 0.81^{a}	4.72 ± 0.14^{b}	5.52 ± 0.13^{a}	12.15 ± 0.13^{a}	8.00 ± 0.10^{b}	0.74 ± 0.24^{a}	0.85 ± 0.01^{a}
Mv	28.91 ± 0.71	43.74 ± 0.18^{b}	45.71 ± 1.47^{a}	202.46 ± 1.07^{b}	227.25 ± 2.74^{a}	39.25 ± 0.60^{b}	42.02 ± 0.70^{a}	32.16 ± 0.51^{b}	39.49 ± 1.00^{a}	3.09 ± 0.02^{b}	4.76 ± 0.16^{a}	198.83 ± 0.07^{b}	242.47 ± 0.51^{a}	40.47 ± 0.55^{b}	44.76 ± 1.03^{a}	108.39 ± 0.51^{b}	121.26 ± 0.51^{a}	2.93 ± 1.03^{b}	4.50 ± 0.01^{a}
Σant	36.89 ± 0.91	52.04 ± 0.30^{b}	55.11 ± 1.66^{a}	245.81 ± 1.58^{b}	268.13 ± 3.62^{a}	47.20 ± 1.01^{b}	51.21 ± 0.82^{a}	43.66 ± 0.92^{b}	52.89 ± 1.25^{a}	5.79 ± 0.24^{b}	7.87 ± 0.23^{a}	239.29 ± 1.19^{b}	291.27 ± 1.76^{a}	48.56 ± 0.93^{b}	54.06 ± 1.24^{a}	134.67 ± 0.86^{b}	142.29 ± 0.99^{a}	5.62 ± 1.29^{b}	7.65 ± 0.13^{a}

Mean (n=3) ± SD. For each yeast strain from the same maceration temperature and stage of fermentation with or without added enzymes, different letters in the same row indicate significant difference at $p < 0.05$. Dp, delphinidin-3-O-glucoside; Cy, cyanidin-3-O-glucoside; Pn, Peonidin-3-O-glucoside; Mv, malvidin-3-O-glucoside; Σant, sum of anthocyanins glycosylated; n.d, not detected values.

Table III.19: Individual anthocyanin concentrations (mg/l) in wines from *Vitis vinifera* L. cv. Cabernet Sauvignon Saint Thomas from the 2015 vintage, at the beginning (T0), middle (T1/2) and final stages of fermentation (TF) of the must macerated at different temperatures with or without added enzymes (70°C, 70°C + enzymes, 25°C and 25°C + enzymes) and fermented with two different yeast strains (X and Y)

	CS-ST-2015																	
	X										Y							
	T0	T0	T0	T1/2	T1/2	T1/2	T1/2	TF	TF	TF	TF	T1/2	T1/2	T1/2	T1/2	TF	TF	TF	TF
	Control	70°C	70°C + Enz	25°C	25°C + Enz	70°C	70°C + Enz	25°C	25°C + Enz	70°C	70°C + Enz	25°C	25°C + Enz	70°C	70°C + Enz	25°C	25°C + Enz	70°C	70°C + Enz
Dp	n.d	2.25 ± 0.25^{a}	2.40 ± 0.15^{a}	5.19 ± 0.00^{b}	6.12 ± 0.01^{a}	2.00 ± 0.71^{a}	2.30 ± 0.53^{a}	4.18 ± 0.09^{b}	5.10 ± 0.00^{a}	1.94 ± 0.44^{a}	2.15 ± 0.22^{a}	5.10 ± 0.02^{a}	6.96 ± 0.10^{a}	2.10 ± 0.52^{b}	2.35 ± 0.04^{a}	4.61 ± 0.03^{b}	6.24 ± 0.14^{a}	2.00 ± 0.03^{a}	2.33 ± 0.40^{a}
Cy	1.28 ± 0.01	n.d	n.d	2.14 ± 0.00^{a}	1.77 ± 0.00^{b}	n.d	n.d	2.16 ± 0.03^{a}	1.62 ± 0.03^{b}	n.d	n.d	1.65 ± 0.04^{a}	1.83 ± 0.12^{a}	n.d	n.d	1.42 ± 0.04^{b}	1.61 ± 0.11^{a}	n.d	n.d
Pn	1.21 ± 0.03	4.49 ± 0.72^{b}	5.53 ± 0.04^{a}	4.81 ± 0.02^{a}	4.85 ± 0.05^{a}	0.92 ± 0.02^{a}	0.90 ± 0.02^{a}	1.84 ± 0.06^{b}	2.31 ± 0.07^{a}	0.65 ± 0.02^{b}	0.75 ± 0.00^{a}	3.97 ± 0.16^{b}	5.21 ± 0.16^{a}	0.93 ± 0.01^{a}	1.10 ± 0.10^{a}	2.86 ± 0.01^{b}	3.63 ± 0.13^{a}	0.77 ± 0.02^{a}	0.95 ± 0.30^{a}
Mv	12.56 ± 0.12	50.28 ± 0.20^{b}	54.83 ± 0.45^{a}	123.52 ± 1.17^{b}	146.27 ± 1.30^{a}	25.35 ± 0.52^{b}	29.29 ± 0.06^{a}	56.21 ± 1.33^{b}	61.65 ± 0.55^{a}	4.74 ± 0.25^{b}	7.11 ± 0.03^{a}	112.96 ± 1.01^{b}	133.56 ± 1.01^{a}	27.80 ± 0.99^{b}	31.04 ± 0.65^{a}	65.93 ± 1.97^{b}	75.73 ± 1.01^{a}	4.27 ± 0.06^{b}	8.38 ± 0.51^{a}
Σant	15.05 ± 0.16	57.02 ± 1.17^{b}	62.76 ± 0.64^{a}	135.66 ± 1.19^{b}	159.01 ± 1.32^{a}	28.27 ± 1.25^{b}	32.49 ± 0.61^{a}	64.39 ± 1.51^{b}	70.68 ± 0.65^{a}	7.33 ± 0.71^{b}	10.01 ± 0.25^{a}	123.68 ± 1.23^{b}	146.56 ± 1.23^{a}	30.83 ± 1.52^{b}	34.49 ± 0.77^{a}	75.22 ± 2.05^{b}	88.01 ± 1.39^{a}	7.04 ± 0.11^{b}	11.66 ± 1.21^{a}

Mean (n=3) ± SD. For each yeast strain from the same maceration temperature and stage of fermentation with or without added enzymes, different letters in the same row indicate significant difference at $p < 0.05$. Dp, delphinidin-3-O-glucoside; Cy, cyanidin-3-O-glucoside; Pn, Peonidin-3-O-glucoside; Mv, malvidin-3-O-glucoside; Σant, sum of anthocyanins glycosylated; n.d, not detected values.

III.7.2. FLAVAN-3-OLS AND NON-FLAVONOIDS PROFILE

Table III.20 and III.21 showed the flavanol, phenolic acid and stilbene concentrations (mg/l) in wines from Syrah and Cabernet Sauvignon during the beginning, middle and end stages of alcoholic fermentation fermented with two different yeast strains. As it can be seen from Table III.20 and III.21, Syrah and Cabernet Sauvignon musts and wines premacerated at 70°C with added enzymes for 24 hours showed the highest total non-anthocyanin content for the two yeast strains. Values for the two grape varieties and yeast strains were approximately 1.17 times higher for wines premacerated at 70°C + enzymes than wines premacerated at 70°C. Similarly to wine samples premacerated at 70°C + enzymes, the control with added enzymes showed an average value 1.29 times higher than the control without added enzymes at the end of alcoholic fermentation. In addition, epigallocatechin was the most abundant flavanol in Syrah and Cabernet Sauvignon musts macerated at 70°C and 70°C + enzymes, while catechin was the most abundant flavanol in must control for the two grape varieties. From control must and must macerated at 70°C and 70°C + enzymes to wine samples (end of alcoholic fermentation) a significant drop in total flavanols content was observed for the two grape varieties and the two yeast strains. Total flavanols drop ranging from 29.30 to 52.98% for must premacerated at 70°C and 70°C + enzymes and from 15.38 to 38.51% for the control (25°C and 25°C + enzymes) for the two grape varieties and X and Y yeast strains. During alcoholic fermentation of wine samples premacerated at 70°C and 70°C + enzymes, certain flavanols compounds showed a significant increment, which is probably as consequence of the hydrolysis that suffer their polymeric and galloylated precursors during fermentation process. For example, the increase observed in epicatechin, and procyanidin B2 could be probably a consequence of the hydrolysis from their galloylated precursors, like epicatechin gallate and procyanidin dimer monogallate respectively. This result is consistent with the statistically increase observed in gallic acid from grape must to wine, these results were supported by previous studies (Lingua et al., 2016). In addition to the increase of epicatechin and procyanidin B2 control showed increase of catechin, Pro B2 and epicatechin gallate. In fact, esterification of epicatechin with gallic acid under the action of esterase during maceration and fermentation may explain the increase of epicatechin gallate during the fermentation of the control.

Concerning non-flavonoid phenolic compounds, with few exceptions, a slight decrease of caffeic acid was observed in wine samples after alcoholic fermentation. CS-70°C + enzymes fermented

with X strain showed the highest concentration (5.68 mg/l). In addition from must to T1/2 of fermentation, ferulic acid showed an increase of content for all wine samples. From T1/2 to wine samples, different trend was observed depending in macerated must temperatures. At temperatures of 70°C and 70°C + enzymes, ferulic acid content decreased from T1/2 to wine, while control showed an increasing. Wine samples with added enzymes showed the highest concentrations ($[FA]_{Sy\text{-}TF\text{-}Y\text{-}70°C+E}$ = 122.80 mg/l and $[FA]_{Sy\text{-}TF\text{-}X\text{-}25°C+E}$ = 99.63 mg/l). Increasing in the concentration of caffeic and ferulic acid comes from the hydrolysis of their ester form caftaric and fertaric acids.

In this study trans-resveratrol was the only detected stilbenes in samples examined. From must to middle stages of fermentation (T1/2), we observed that this compound increased significantly. From T1/2 to wine samples, this compound did not follow a common trend for the different premacerated temperatures. For wine premacerated at 70°C and 70°C + enzymes a decrease in concentration was observed, whereas for wine control an increase of content was revealed. Wines fermented with added enzymes showed the highest value ($[Res]_{Sy\text{-}TF\text{-}Y\text{-}70°C+E}$ = 19.96 mg/l and ($[Res]_{Sy\text{-}TF\text{-}Y\text{-}25°C+E}$ = 15.70 mg/l). Actually the absorption of resveratrol by yeast cells has been observed (Barcia et al., 2014b) as well as, hydrolysis of it is glucoside and *cis/trans* isomerization has also been reported during winemaking (Monagas et al., 2005b). Therefore according to these factors, the first could be explaining the reduction of *trans*-resveratrol content in the finished wines. In case of control, the second factor would be prevailing over the first. After all, our results indicated that wines fermented with adding enzymes contain higher concentration of phenolic compounds than wines fermented without added enzymes. As a matter of fact and as seen previously, pectolytic enzymes breakdown berry cell wall structural components and therefore favors higher extraction of phenolic compounds.

Table III.20: Individual non-anthocyanin phenolic compounds (mg/l) in wines from *Vitis vinifera* cv. Syrah Saint Thomas from the 2015 vintage at the beginning (T0), middle (T1/2) and final stages of fermentation (TF) of the must macerated at different temperatures with or without added enzymes (70°C, 70°C + enzymes, 25°C and 25°C + enzymes) and fermented with two different yeast strains (X and Y)

				Sy-ST-2015															
				X								Y							
	T0	T0	T0	T1/2	T1/2	T1/2	T1/2	TF	TF	TF	TF	T1/2	T1/2	T1/2	T1/2	TF	TF	TF	TF
	Control	70°C	70°C + Enz	25°C	25°C + Enz	70°C	70°C + Enz	25°C	25°C + Enz	70°C	70°C + Enz	25°C	25°C + Enz	70°C	70°C + Enz	25°C	25°C + Enz	70°C	70°C + Enz
Flavanols																			
(+)- Cat	19.53 ± 0.63	112.78 ± 0.49^{b}	120.48 ± 1.10^{a}	43.88 ± 0.76^{a}	41.01 ± 0.69^{b}	86.54 ± 3.39^{a}	70.98 ± 1.78^{b}	51.52 ± 0.41^{a}	52.39 ± 0.99^{a}	10.40 ± 0.41^{a}	6.67 ± 0.21^{b}	46.30 ± 0.54^{b}	52.07 ± 0.68^{a}	87.04 ± 1.39^{b}	90.05 ± 1.96^{a}	51.95 ± 0.34^{b}	62.65 ± 0.13^{a}	31.71 ± 0.18^{b}	56.19 ± 0.71^{a}
(-)- Epi	11.79 ± 0.40	145.76 ± 0.53^{a}	161.52 ± 1.23^{b}	79.60 ± 1.20^{a}	73.20 ± 1.24^{b}	193.74 ± 0.64^{b}	216.79 ± 2.67^{a}	72.07 ± 0.96^{b}	78.91 ± 0.41^{a}	61.19 ± 0.45^{b}	200.80 ± 0.20^{a}	81.25 ± 0.28^{b}	92.70 ± 0.53^{a}	214.90 ± 2.95^{b}	245.70 ± 0.25^{a}	88.56 ± 0.76^{b}	98.83 ± 0.31^{a}	118.89 ± 0.60^{b}	176.34 ± 0.83^{a}
(-)- Epig	5.50 ± 0.22	53.27 ± 0.14^{a}	54.32 ± 0.15^{a}	16.83 ± 0.28^{b}	25.94 ± 1.21^{a}	32.56 ± 0.27^{a}	31.10 ± 0.12^{a}	20.77 ± 0.32^{b}	23.61 ± 0.32^{a}	19.24 ± 0.20^{a}	20.14 ± 0.74^{a}	18.21 ± 0.12^{b}	20.21 ± 0.34^{a}	30.27 ± 0.74^{b}	32.22 ± 0.21^{a}	20.52 ± 0.89^{a}	19.36 ± 0.54^{a}	20.12 ± 0.13^{b}	21.25 ± 0.65^{a}
(-)- EpiG	18.44 ± 0.52	340.95 ± 0.03^{a}	314.51 ± 0.14^{b}	71.82 ± 0.93^{b}	81.74 ± 0.40^{a}	221.57 ± 0.03^{a}	214.75 ± 0.17^{b}	64.36 ± 0.26^{a}	64.71 ± 0.82^{a}	200.24 ± 0.07^{a}	192.85 ± 0.04^{b}	80.41 ± 0.62^{b}	92.90 ± 0.71^{a}	286.40 ± 0.08^{b}	294.67 ± 0.19^{a}	66.37 ± 0.29^{b}	80.42 ± 0.72^{a}	245.16 ± 0.01^{a}	243.32 ± 0.01^{b}
Pro B1	14.63 ± 0.32	156.46 ± 1.97^{a}	155.17 ± 1.49^{a}	15.08 ± 0.46^{b}	16.65 ± 0.06^{a}	77.27 ± 0.33^{b}	86.45 ± 0.39^{a}	41.93 ± 0.61^{b}	46.23 ± 0.64^{a}	46.13 ± 0.06^{b}	57.41 ± 0.31^{a}	19.11 ± 0.73^{a}	20.86 ± 0.71^{a}	107.79 ± 1.01^{b}	115.29 ± 1.96^{a}	14.50 ± 0.28^{b}	118.88 ± 0.84^{a}	68.31 ± 0.96^{b}	71.37 ± 0.31^{a}
Pro B2	64.15 ± 0.55	157.73 ± 1.47^{b}	176.00 ± 1.40^{a}	236.58 ± 0.18^{b}	274.40 ± 1.01^{a}	162.33 ± 3.69^{b}	175.69 ± 1.71^{a}	63.09 ± 0.89^{b}	96.44 ± 0.39^{a}	124.73 ± 0.65^{b}	130.42 ± 0.06^{a}	291.67 ± 0.85^{b}	319.02 ± 0.08^{a}	142.74 ± 0.85^{b}	158.05 ± 1.91^{a}	88.26 ± 0.32^{b}	125. 68 ± 0.61^{a}	103.34 ± 1.38^{b}	125.75 ± 0.49^{a}
Σflavanols	134.04 ± 2.64	966.95 ± 4.63^{b}	982.00 ± 5.37^{a}	463.79 ± 3.81^{a}	512.94 ± 4.61^{b}	774.01 ± 8.35^{b}	795.76 ± 6.84^{a}	313.74 ± 3.45^{b}	362.29 ± 3.57^{a}	461.93± 1.84^{b}	608.29 ± 1.56^{a}	536.95 ± 3.10^{b}	597.76 ± 2.34^{a}	869.14 ± 7.02^{b}	935.98 ± 6.48^{a}	330.16 ± 2.88^{b}	505.82 ± 3.15^{a}	587.53 ± 3.26^{b}	694.22 ± 3.00^{a}
Phenolic acids																			
Gallic acid	3.85 ± 0.03	38.49 ± 0.56^{b}	51.29 ± 0.48^{a}	16.62 ± 0.17^{a}	15.81 ± 0.15^{b}	48.31 ± 0.68^{b}	56.67 ± 0.84^{a}	23.92 ± 0.51^{a}	21.81 ± 0.85^{b}	54.48 ± 0.26^{b}	61.36 ± 0.01^{a}	18.90 ± 0.79^{a}	19.35 ± 0.71^{a}	45.06 ± 0.71^{b}	58.73 ± 0.61^{a}	23.21 ± 0.10^{b}	26.88 ± 0.52^{a}	52.78 ± 0.72^{b}	60.57 ± 0.05^{a}
Caffeic acid	2.84 ± 0.02	6.88 ± 0.08^{a}	6.62 ± 0.01^{b}	4.44 ± 0.22^{a}	4.35 ± 0.18^{a}	5.71 ± 0.15^{a}	5.63 ± 0.28^{a}	3.12 ± 0.11^{a}	3.04 ± 0.11^{a}	4.92 ± 0.07^{a}	4.69 ± 0.24^{a}	4.75 ± 0.21^{a}	4.74 ± 0.18^{a}	5.74 ± 0.03^{b}	6.37 ± 0.11^{a}	3.43 ± 0.15^{a}	3.20 ± 0.09^{a}	5.59 ± 0.07^{a}	5.62 ± 0.14^{a}
Ferulic acid	8.54 ± 0.32	66.26 ± 0.61^{b}	72.25 ± 0.17^{a}	82.48 ± 0.58^{b}	86.52 ± 0.39^{a}	106.51 ± 0.27^{a}	101.76 ± 0.60^{b}	77.86 ± 0.95^{b}	99.63 ± 0.26^{a}	68.16 ± 0.45^{b}	82.20 ± 0.83^{a}	26.85 ± 0.56^{a}	15.70 ± 0.74^{b}	123.96 ± 0.71^{a}	122.17 ± 0.78^{b}	55.62 ± 0.40^{a}	50.93 ± 0.73^{b}	63.26 ± 1.01^{b}	122.80 ± 0.17^{a}
Stilbenes																			
Resveratrol	2.94 ± 0.02	1.96 ± 0.01^{a}	1.95 ± 0.00^{a}	6.58 ± 0.22^{a}	6.73 ± 0.03^{a}	18.40 ± 0.06^{a}	15.84 ± 0.25^{b}	10.55 ± 0.24^{a}	10.78 ± 0.06^{a}	10.46 ± 0.01^{b}	12.09 ± 0.08^{a}	7.43 ± 0.01^{b}	7.78 ± 0.25^{a}	19.58 ± 0.51^{b}	21.99 ± 0.01^{a}	6.46 ± 0.01^{b}	15.70 ± 0.57^{a}	17.54 ± 0.21^{b}	19.96 ± 0.01^{a}
Σ total non-ant	152.21 ± 3.03	1080.54 ± 5.89^{b}	1114.11 ± 6.09^{a}	567.33 ± 5.00^{b}	626.35 ± 5.36^{a}	952.94 ± 9.51^{b}	975.66 ± 8.81^{a}	429.19 ± 5.26^{b}	497.55 ± 4.85^{a}	599.95 ± 2.63^{b}	768.63 ± 2.12^{a}	594.88 ± 4.71^{b}	645.33 ± 4.93^{a}	1063.48 ± 8.99^{b}	1145.24 ± 7.89^{a}	418.88 ± 3.54^{b}	602.53 ± 5.06^{a}	726.80 ± 5.36^{b}	903.17 ± 3.43^{a}

Mean (n=3) ± SD. For each yeast strain from the same maceration temperature and stage of fermentation with or without added enzymes different letters in the same row indicate significant difference at $p < 0.05$. Cat, catechin; Epi, epicatechin; Epig, epicatechin gallate; EpiG, epigallocatechin; Pro B1, procyanidin B1; Pro B2, procyanidin B2; Σ total non-ant, sum of total non anthocyanins

Table III.21: Individual non-anthocyanin phenolic compounds (mg/l) in wines from *Vitis vinifera* cv. Cabernet Sauvignon Saint Thomas from the 2015 vintage at the beginning (T0), middle (T1/2) and final stages of fermentation (TF) of the must macerated at different temperatures with or without added enzymes (70°C, 70°C + enzymes, 25°C and 25°C + enzymes) and fermented with two different yeast strains (X and Y)

				CS-ST-2015															
				X								Y							
	T0	T0	T0	T1/2	T1/2	T1/2	T1/2	TF	TF	TF	TF	T1/2	T1/2	T1/2	T1/2	TF	TF	TF	TF
	Control	70°C	70°C + Enz	25°C	25°C + Enz	70°C	70°C + Enz	25°C	25°C + Enz	70°C	70°C + Enz	25°C	25°C + Enz	70°C	70°C + Enz	25°C	25°C + Enz	70°C	70°C + Enz
Flavanols																			
(+)- Cat	3.17 ± 0.17	86.12 ± 4.05^{b}	128.63 ± 0.67^{a}	37.39 ± 0.08^{b}	38.36 ± 0.33^{a}	60.89 ± 0.69^{b}	84.30 ± 3.09^{a}	44.70 ± 1.31^{b}	49.55 ± 1.05^{a}	50.76 ± 1.27^{b}	75.92 ± 0.65^{a}	41.59 ± 0.68^{b}	59.27 ± 0.85^{a}	83.01 ± 0.45^{b}	108.17 ± 0.91^{a}	44.42 ± 1.00^{b}	68.12 ± 1.24^{a}	79.39 ± 0.53^{b}	97.21 ± 0.88^{a}
(-)- Epi	3.34 ± 0.04	114.47 ± 1.28^{a}	108.03 ± 0.35^{b}	60.30 ± 0.56^{b}	63.41 ± 1.00^{a}	146.96 ± 0.33^{b}	159.16 ± 4.43^{a}	56.57 ± 1.49^{b}	61.00 ± 0.70^{a}	113.02 ± 1.74^{a}	114.41 ± 2.92^{a}	74.10 ± 0.26^{b}	80.97 ± 0.73^{a}	114.46 ± 2.98^{b}	131.34 ± 0.30^{a}	83.80 ± 1.06^{b}	90.28 ± 1.00^{a}	105.21 ± 0.76^{a}	107.84 ± 0.75^{a}
(-)- Epig	2.13 ± 0.10	30.21 ± 2.55^{a}	30.46 ± 2.32^{a}	9.19 ± 0.20^{a}	9.69 ± 0.08^{a}	23.15 ± 0.74^{b}	30.10 ± 0.31^{a}	22.53 ± 0.24^{b}	24.48 ± 0.29^{a}	20.10 ± 0.13^{b}	28.40 ± 1.07^{a}	7.48 ± 0.11^{b}	10.70 ± 0.95^{a}	25.45± 1.27^{b}	30.16 ± 0.22^{a}	23.75 ± 0.84^{b}	32.05 ± 2.29^{a}	22.03 ± 0.24^{b}	28.42 ± 1.23^{a}
(-)- EpiG	4.60 ± 0.24	434.11 ± 0.75^{a}	431.95 ± 0.85^{b}	55.42 ± 0.53^{b}	62.81 ± 0.24^{a}	332.51 ± 0.05^{a}	325.62 ± 0.00^{b}	41.98 ± 0.75^{b}	45.73 ± 0.35^{a}	208.66 ± 0.93^{a}	193.57 ± 2.34^{b}	48.22 ± 0.37^{b}	71.01 ± 0.75^{a}	385.50 ± 0.01^{a}	384.93 ± 0.51^{a}	22.74 ± 0.58^{b}	55.53 ± 0.08^{a}	275.55 ± 0.60^{a}	265.56 ± 1.12^{b}
Pro B1	2.77 ± 0.04	236.68 ± 4.14^{b}	387.78 ± 0.76^{a}	15.07 ± 0.37^{a}	15.60 ± 0.11^{a}	132.36 ± 2.39^{b}	146.75 ± 0.64^{a}	30.12 ± 0.53^{b}	36.40 ± 0.75^{a}	122.01 ± 3.10^{b}	128.19 ± 0.44^{a}	14.58 ± 0.09^{b}	17.53 ± 0.20^{a}	173.01 ± 1.19^{a}	156.34 ± 2.29^{b}	72.09 ± 1.58^{b}	134.60 ± 1.63^{a}	135.50 ± 0.28^{a}	117.07 ± 1.91^{b}
Pro B2	2.26 ± 0.02	251.28 ± 0.55^{a}	337.46 ± 0.58^{b}	200.90 ± 0.83^{b}	220.34 ± 0.56^{a}	135.51 ± 4.19^{b}	153.73 ± 0.97^{a}	90.81 ± 0.45^{b}	100.41 ± 0.48^{a}	127.28 ± 0.52^{b}	129.21 ± 0.92^{a}	256.71 ± 1.81^{b}	308.86 ± 1.70^{a}	142.51 ± 0.93^{a}	141.91 ± 1.52^{a}	93.55 ± 1.05^{b}	110.63 ± 0.45^{a}	111.97 ± 0.31^{b}	116.53 ± 0.27^{a}
Σflavanols	18.27 ± 0.61	1152.87 ± 13.32	1424.31 ± 5.53^{a}	378.27 ± 2.57^{b}	410.21 ± 2.32^{a}	831.38 ± 8.39^{b}	899.66 ± 9.44^{a}	286.71 ± 4.77^{b}	317.57 ± 3.62^{a}	641.83 ± 7.69^{b}	669.70 ± 8.34^{a}	442.68 ± 3.32^{b}	548.34 ± 5.18^{a}	923.94 ± 6.83^{b}	952.85 ± 5.75^{a}	340.35 ± 6.11^{b}	491.21 ± 6.69^{a}	729.65 ± 2.72^{b}	732.63 ± 6..16^{a}
Phenolic acids																			
Gallic acid	1.43 ± 0.00	47.77 ± 0.71^{b}	52.32 ± 0.38^{a}	12.02 ± 0.15^{a}	12.26 ± 0.56^{a}	50.50 ± 0.29^{b}	58.58 ± 1.25^{a}	28.03 ± 0.94^{a}	24.43 ± 0.18^{b}	57.19 ± 1.03^{b}	67.16 ± 2.36^{a}	10.36 ± 0.20^{b}	11.44 ± 0.28^{a}	52.43 ± 0.35^{b}	56.80 ± 0.57^{a}	20.43 ± 0.17^{b}	25.83 ± 0.45^{a}	63.60 ± 0.80^{b}	74.79 ± 0.32^{a}
Caffeic acid	2.56 ± 0.02	4.11 ± 0.14^{a}	3.82 ± 0.15^{b}	2.85 ± 0.06^{a}	2.85 ± 0.04^{a}	4.32 ± 0.17^{b}	6.61 ± 0.18^{a}	2.56 ± 0.01^{a}	2.63 ± 0.12^{a}	4.05 ± 0.02^{b}	5.68 ± 0.46^{a}	2.71 ± 0.06^{a}	2.86 ± 0.16^{a}	5.04 ± 0.03^{b}	6.08 ± 0.06^{a}	2.69 ± 0.09^{a}	2.81 ± 0.26^{a}	4.63 ± 0.03^{b}	5.64 ± 0.49^{a}
Ferulic acid	3.26 ± 0.04	29.15 ± 2.85^{b}	31.45 ± 0.91^{a}	23.61 ± 0.13^{a}	24.51 ± 0.94^{a}	130.48 ± 0.20^{b}	178.16 ± 1.30^{a}	30.14 ± 0.96^{b}	57.43 ± 0.45^{a}	65.94 ± 0.36^{b}	92.26 ± 0.67^{a}	19.95 ± 0.93^{b}	24.24 ± 0.84^{a}	112.46 ± 0.39^{b}	117.64 ± 0.16^{a}	27.81 ± 0.14^{b}	71.60 ± 1.00^{a}	44.55 ± 0.03^{b}	70.86 ± 1.24^{a}
Stilbenes																			
Resveratrol	1.95 ± 0.00	4.87 ± 0.02^{a}	4.48 ± 0.09^{a}	6.43 ± 0.04^{a}	3.15 ± 0.05^{b}	14.18 ± 0.14^{b}	19.54 ± 0.07^{a}	6.56 ± 0.11^{b}	9.52 ± 0.00^{a}	10.31 ± 0.05^{b}	14.36 ± 0.58^{a}	5.96 ± 0.06^{a}	5.76 ± 0.15^{a}	15.08 ± 0.36^{b}	19.50 ± 0.44^{a}	6.19 ± 0.10^{b}	11.13 ± 0.89^{a}	14.67 ± 0.35^{a}	14.58 ± 0.44^{a}
Σ total non-ant	27.47 ± 0.67	1238.77 ± 17.04^{b}	1516.38 ± 7.06^{a}	423.18 ± 2.95^{b}	452.98 ± 3.91^{a}	1030.86 ± 8.45^{b}	1162.55 ± 12.68^{a}	354.00 ± 6.79^{b}	411.58 ± 4.27^{a}	779.32 ± 9.15^{b}	849.16 ± 14.55^{a}	481.66 ± 4.55^{b}	592.64 ± 6.61^{a}	1108.95 ± 6.72^{b}	1152.87 ± 9.41^{a}	397.47 ± 6.61^{b}	602.58 ± 9.74^{a}	857.10 ± 3.93^{b}	898.50 ± 8.65^{a}

Mean (n=3) ± SD. For each yeast strain from the same maceration temperature and stage of fermentation with or without added enzymes different letters in the same row indicate significant difference at $p < 0.05$. Cat, catechin; Epi, epicatechin; Epig, epicatechin gallate; EpiG, epigallocatechin; Pro B1, procyanidin B1; Pro B2, procyanidin B2; Σ total non-ant, sum of total non anthocyanins.

III.8. Biological activities

According to the antioxidant (ABTS and DPPH), anti-inflammatory (LOX), anticancer (cytotoxicity) and antidiabetic (α-glucosidase) activities which has been associated to the polyphenol content of wine (Halpern 2008), Figure III.4 and III.5 present respectively the comparative biological activities (ABTS, DPPH, LOX, α-glucosidase, ChE and XOD) of Syrah and Cabernet Sauvignon Saint Thomas of 2015 vintage compared to control conditions at the beginning and the final stages of alcoholic fermentation. For the two grape varieties, must macerated at 70°C after 24 hours possess many biological activities with the highest inhibition‟s percentage (ABTS, DPPH, LOX, α-glucosidase and ChE), compared to must macerated at 60°C (ABTS and DPPH) and the control (ABTS and LOX), with almost nonexistent biological activities. After alcoholic fermentation, almost all of the wine samples presented an increase of their percentage of inhibition (except for Sy-70°C whose ABTS and DPPH activities showed a decrease in their inhibition‟s percentage after fermentation) with the occurrence of new types of biological activities which doesn‟t existed at must level. Syrah and Cabernet Sauvignon 60°C fermented wines by the two yeast strains showed the same biological activity profiles with different biological activities potential. The strongest inhibitory activity was observed for CS; values for CS-60°C-Y strain were 1.2; 1.2; 5.3; 1.5 times much higher respectively for ABTS, DPPH, LOX and α-glucosidase than for Syrah for the same yeast strain and the same activities. Y strain showed the highest inhibitory activity for the two grape varieties (except for Syrah anti-LOX activity). CS-70°C-Y fermented wines showed significantly (Figure III.4 and III.5) higher antioxidant activities (+7% for ABTS and +10% for DPPH), anti-LOX (+36%) and anti-α-glucosidase (+56%) activities than for Syrah Y fermented wines at the same temperature. A slight inhibition percentage of anti-CHE activity (5.17%) was observed by Sy-70°C-Y fermented wines. Moreover, CS-70°C-X fermented wines showed higher antioxidant and anti-α-glucosidase activities than Sy-70°C-X fermented wines, values were 35.04; 19.17 and 77.4% respectively for ABTS, DPPH and anti-α-glucosidase, whereas Sy-70°C-X showed the highest anti-LOX activity (56.61%). Regarding Syrah Y fermented wines, As seen in Figure III.4, the control with added enzymes (25°C-Y + enzymes) had the highest percentage of inhibition for all of the biological activities studied, on which anti-LOX and anti-α-glucosidase showed respectively maximum inhibitory activity of 82.14 and 95.34%. In order to better evaluate the importance of the inhibition percentage, the biological activities were repeated at a final concentration of 100 mg/l

(Figure III.6) of wine extract in microplate, in samples which had an inhibition percentage greater than or equal to 80% (at 500 mg/l). Figure III.6 showed that Sy-25°C-Y+ enzymes had the highest inhibition percentage of 53.98% of antidiabetic activities, followed by Sy-25°C-X (24.12%) and Sy-25°C-X + enzymes (20.66%). Moreover, at final concentration of 100 mg/l of wine extract Sy-25°C-Y + enzymes exhibited an inhibition percentage of 41.96% for anti-LOX activities (data not shown). In opposition to Syrah, CS-70°C-Y fermented wines showed slightly higher inhibition percentage for the biological activities analyzed than for CS 25°C-Y + enzymes (Figure III.5).

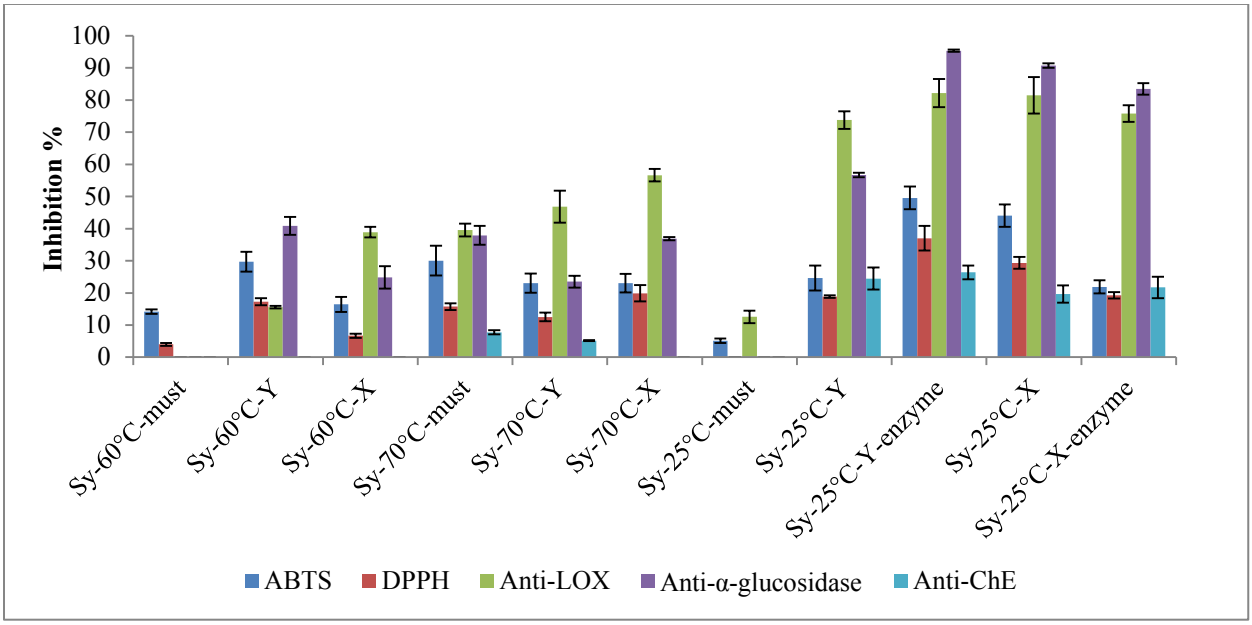

Figure III.4: Biological activities (ABTS and DPPH (antioxidant), Anti-LOX (antiinflammatory), Anti-α glucosidase (antidiabetic) and Anti-ChE (antialzheimer)) of Sy (Syrah) grape musts and wines premacerated at different temperatures for 24 hours (60°C and 70°C) compared to the control musts and wines with and without added enzymes (classic vinification, 25°C and 25°C + enzymes) and fermented by two yeast strains (X and Y). Data were expressed as mean percentage of inhibition (inhibition %) ± standard deviation.

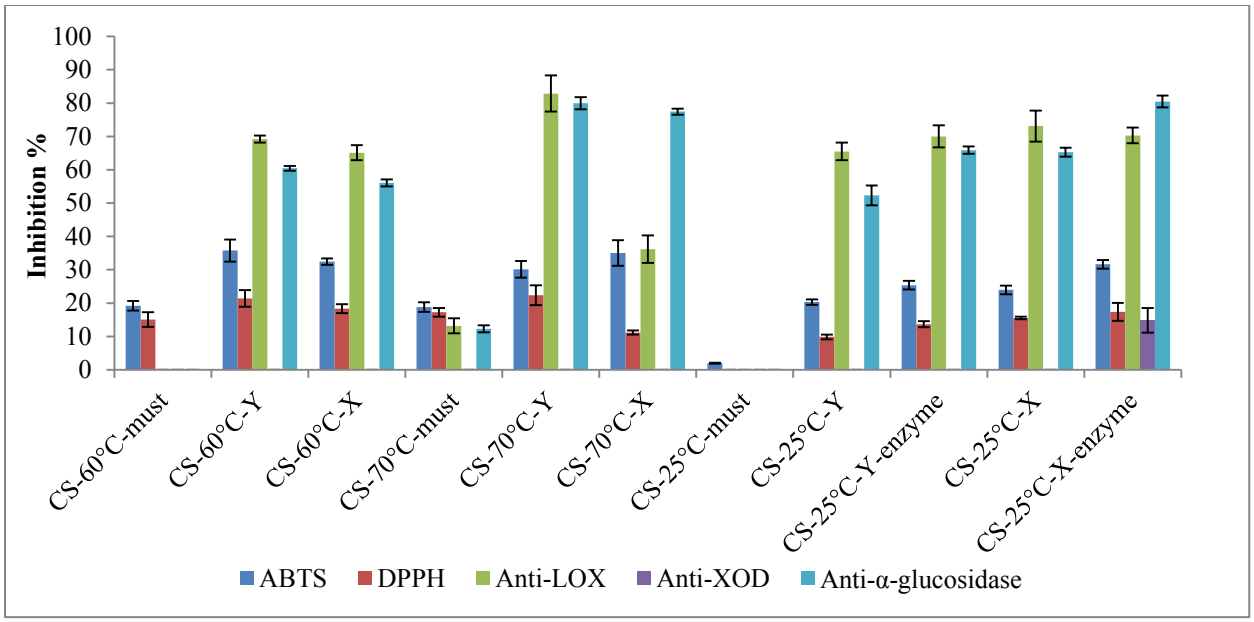

Figure III.5: Biological activities (ABTS and DPPH (antioxidant), Anti-LOX (antiinflammatory), Anti-XOD (anti-hyperuricemic) and Anti-α glucosidase (antidiabetic)) of CS (Cabernet Sauvignon) grape musts and wines premacerated at different temperatures for 24 hours (60°C and 70°C), compared to the control musts and wines with and without added enzymes (classic vinification, 25°C and 25°C + enzymes) and fermented by two yeast strains (X and Y). Data were expressed as mean percentage of inhibition (inhibition %) ± standard deviation

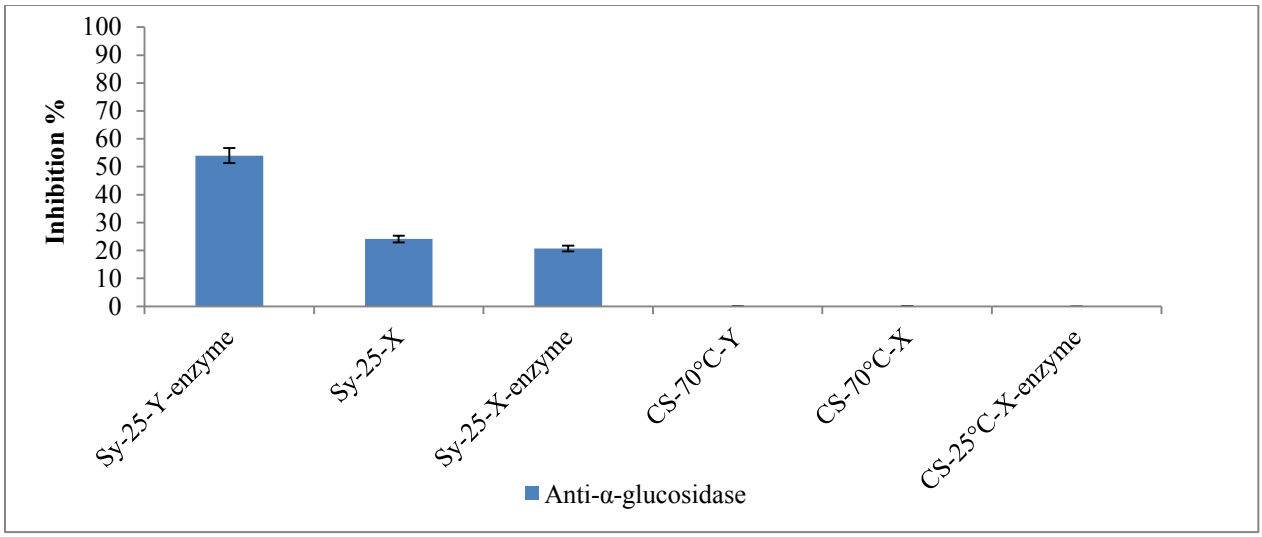

Figure III.6: comparison of Anti-α-glucosidase activity for Sy (Syrah) and CS (Cabernet Sauvignon) control wines (at the end of alcoholic fermentation) with or without enzymes (25°C/25°C + enzymes) and for CS wine premacerated at 70°C and fermented by the two yeast strains (Y and X) at final concentration of 100 mg/l of wine extract in microplate wells. Data were expressed as mean percentage of inhibition (inhibition %) ± standard deviation

In order to better assess which phenolic compound contribute the most for the different biological activities of Syrah and Cabernet Sauvignon musts and wines, principal component analysis was performed. Figure III.7, showed the PCA biplot for the first two principal component analyses which explain 71.47% of the total variance. The first component is positively represented by the variables TPI, TP, T, GA, Pro B1, EpiG, Cat, Pro B2, Epig, Epic and CA. The second component is positively represented by TA, Dp, Cy, Pn and Mv. The projection of Syrah and Cabernet Sauvignon must samples over fermentation stages (T0 and TF) at different temperatures with and without enzymes (70°C and 25°C), indicated that Sy-25°C+E-Y (Figure III.7, c) contain higher content in TA, Dp, Pn and Mv which it could explain the importance of their antidiabetic (Figure III.6) and anti-inflammatory activities at final concentration of 100 mg/l. In fact, anthocyanins have been show to inhibit hyperglycemia (type II), improve beta-cell function and protect against beta-cell lost (Zunino, 2009) and They also reduce inflammatory inducers of tumor initiation (Renaud and de Lorgeril, 1992), Moreover, the higher content in GA and CA of CS-70°C-Y (Figure III.7) may could explain their higher inhibition percentage for anti-LOX and anti-α-glucosidase activities compared to CS-25°C-E-Y. In fact studies conducted by (Jung et al., 2007;

Yagi and Ohishi, 1979) has shown that phenolics acids had hypoglycemic and anti-inflammatory effects.

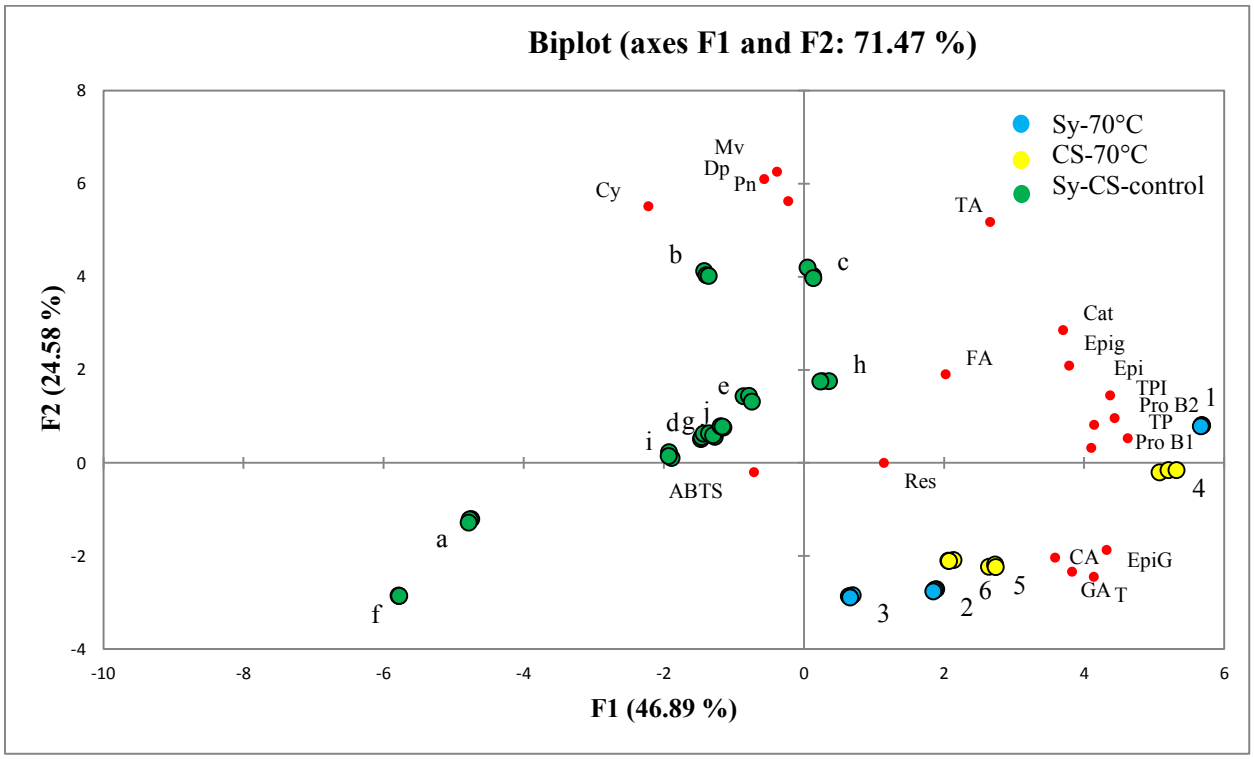

Figure III.7: Biplot of the two first principal components obtained from the antioxidant activities (ABTS) and phenolic composition of Syrah (Sy) and cabernet Sauvignon (CS) musts and wines (at the beginning, T0 and the end, TF of alcoholic fermentation) from the 2015 vintage: TA, total anthocyanin content; TPI, total polyphenol index; TP, total polyphenols; T, Tannins; Dp, delphinidin-3-O-glucoside ; Cy, cyanidin-3-O-glucoside; Pn, peonidin-3-O-glucoside; Mv, malvidin-3-O-glucoside; GA, gallic acid; pro B1, procyanidin B1; EpiG, epigallocatechin; Cat, catechin; Pro B2, procyanidin B2; CA, caffeic acid; Epi, epicatechin; Epig, epicatechin gallate obtained after maceration of the must at 70°C for 24 hours compared to the control with and without added enzymes (classic winemaking, 25°C and 25°C + enzymes) and fermented with two different yeast strains X and Y (a, Sy-25°C-T0; b, Sy-25°C-TF-Y ; c, Sy-25°C-E-TF-Y; d, Sy-25°C-TF-X; e, Sy-25°C-E-TF-X, f, CS-25°C-T0; g, CS-25°C-TF-Y ; h, CS-25°C-E-TF-Y; i, CS-25°C-TF-X; j, CS-25°C-E-TF-X, 1, Sy-70°C-T0-Y; 2, Sy-70°C-TF-Y; 3, Sy-70°C-TF-X; 4, CS-70°C-T0-Y; 5, CS-70°C-TF-Y; 6, CS-70°C-TF-X

III.9. Conclusion

A detailed study on the influence of *S. cerevisiae* yeast strains (X and Y) on the analyses of phenolic compounds of red wines has been conducted. Wines fermented by Y strain showed higher amounts of total anthocyanins compared to those fermented by X strain, whereas this latter showed higher total phenolic compounds suggesting more β-glucosidase activity and high hydrophilic parietal constituents. After alcoholic fermentation, the total polyphenol level in all wines decreased significantly. The main changes observed was an increase of some flavanols and non flavanols (catechin, epicatechin, procyanidin B1 and B2, gallic acid, caffeic and ferulic acids) contents which is probably the consequence of hydrolysis that suffer their polymeric, galloylated precursors and tartaric acid esters during winemaking process. Wine samples with pectolytic enzymes added demonstrated the highest anthocyanin and tannin contents. Results from discriminant analyses revealed that glycosylated delphinidin was the anthocyanin most affected by the yeast strain while Procyanidin B2 was the most affected tannin due to grape varieties. Biological activities analyses showed that after alcoholic fermentation almost all of the wine samples presented an increase of their percentage of inhibition with the occurrence of new types of biological activities which doesn‟t exist at must level. After all, results from PCA revealed that TA, CA and GA could be the most responsible for the strongest antidiabetic and anti-inflammatory effects.

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Chapter IV- Impact of Fining Agents

IV.1. Introduction

Clarity or limpidity is one of the leading consumer quality requirements. It is an important aspect of a consumer‟s first contact with a wine and a key element in visual satisfaction. Particles in suspension, either in forming a haze or dispersed through the liquid, not only spoil the presentation but also affect the tasting (Ribéreau-Gayon et al., 2006). A suitable wine stabilization and limpidity is progressively obtained after winemaking due to physical and chemical phenomena that determine the precipitation of unstable compounds. Stabilization could be divided into physico-chemical and microbiological stabilization. Physico-chemical stabilization, insured by fining agents, prevents the formation of hazes and deposits after bottling while microbiological stabilization is guaranteed by filtration that eliminates yeasts and bacteria (El Rayess et al., 2011).

Fining agents are used to eliminate or reduce undesirable substances in wine. Electrostatic interactions, chemical bond formation and absorption/adsorption are the three major mechanisms of action of fining agents (Ghanem et al., 2014). Fining is responsible for elimination of some phenolic compounds of colloidal nature that can be perceived as improvement of wine characteristics or deterioration of wines if phenolic compounds are excessively removed.

Phenolic compounds are one of the most important quality parameters in red wines, and involve two main groups of compounds, non-flavonoids (hydroxybenzoic and hydroxycinnamic acids and their derivatives and stilbenes) and flavonoids (anthocyanins, flavanols, flavonols, and dihydroflavonols). These compounds contribute to organoleptic characteristics of wines such as color, bitterness and astringency as well as other mouth-feel properties (Oberholster et al., 2009). The phenolic composition of red wines is affected by the wine-making process (Sun et al., 2001). An important step in winemaking is the addition of fining agents, exogenous tannins and commercial mannoproteins.

Several fining agents (bentonite, casein, gelatin, isinglass, polyvinylpolypyrrolidone, etc) are used by winemakers and the choice depends on the compounds that need to be removed. They can be used separately and combined with each other in a defined dosage. Bentonite is mainly negatively-charged clay of volcanic origin with complex hydrated aluminium silicate components. In principle, it is used to remove proteins, thus providing better clarity and stability during long term storage. However, it also attracts other positively charged compounds, such as anthocyanins, other phenolics and nitrogen. It is not reactive towards small phenolic compounds.

In fact, it binds large phenolic compounds, such as anthocyanins, and may also bind phenolic compounds complexed with proteins (Threlfall et al., 1999). Egg albumin, casein, gelatine and PvPP (polyvinylpolypyrrolidone) reduce the phenolic content of wines and may decrease the color of some wines (Castillo-Sanchez et al., 2006). Additionally, in response to winemaker"s interest in finding alternatives to animal proteins for use as fining agents in, a wide variety of commercial preparations of plant-derived proteins from soy, gluten wheat, rice, potato, lupine or maize had been proposed for oenological use with the name of vegetable proteins (Bindon and Smith, 2013). Moreover, some of these plant proteins may precipitate galloylated and condensed tannins depending on their origin and their molecular weight (Maury et al., 2003).

Mannoproteins are one of the major polysaccharide groups present in wine (Feuillat, 2003), derived from the cell wall of *Saccharomyces cerevisiae*, and are increasingly being added in oenological products to wines with the intention of preventing tartaric and protein precipitation (Moine-Ledoux and Dubourdieu, 2002). The interaction between mannoproteins and wine phenolic compounds is a subject of great interest. Studies showed the possible impact on color stability (Escot et al., 2001), an improvement in the sensory characteristics, namely the reduction of red wine astringency (Guadalupe et al., 2007; Poncet-legrand et al., 2007) and improvement of wine aromatic profile (Chalier et al., 2007). In order to prevent oxidation in must made from botrytized grapes, strengthen the wine structure and facilitate ageing, exogenous tannins can be added. The use of oenological tannins may contribute to improve wine color and its stability. Some of the positive effects of using enological tannins include wine color stabilization, improved wine structure, and the control of laccase activity and an elimination of reduction odors (Zamora, 2003). However, other studies showed (Baustita-Ortín, et al., 2005) that the use of enological tannins should be treated with great care, because when used in inappropriate conditions, wines may lose their equilibrium. This effect was more accused when hydrolysable tannins were used.

In this context the aim of this study was to evaluate the effect of five different oenological fining practices (egg albumin, PVPP + casein, bentonite, gelatin and vegetable proteins) and two oenological additives (tannins and mannoproteins); as well as the effect of different fining concentrations on the chromatic characteristics, phenolic composition, and antioxidant activity of Cabernet Sauvignon red wine.

IV.2. Materials and methods

IV.2.1. CHEMICALS AND FINING AGENTS

All chemicals used were of analytical reagent grade. All chromatographic solvents (acetonitrile, acetic acid) were high-performance liquid chromatography (HPLC) grade. Delphinidin 3-O-glucoside, cyanidin 3-O-glucoside, peonidin-3-O-glucoside, malvidin 3-O-glucoside, (+) - Catechin, (-) – Epicatechin, (-) – Epicatechingallate (-) - Epigallocatechin, (-) - Epigallocatechingallate, Procyanidin B1, Procyanidin B2, Ferulic acid, Caffeic acid and trans-resveratrol were purchased from Extrasynthese (Genay, France). The fining agents Ovoclaryl® (egg albumin), Polylact® (PvPP + casein+cellulose), Microcol alpha® (bentonite), Vegecoll® (vegetable protein from potatoe), Gecoll supra® (gelatin), oenological condensed tannins (procyanidin tannin) and wine stabilization Mannostab® (mannoprotein) were purchased from Laffort.

IV.2.2. WINE TREATMENTS

Cabernet Sauvignon wine (pH 3.4, titratable acidity (TA) 3.53g/l as sulphuric acid, residual sugar 1.8 g/l) from the 2014 vintage was provided from Lebanese winery (Clos St. Thomas). This wine was made using classical commercial winemaking process and was obtained after the completion of malolactic fermentation. Fining procedures were conducted for 48 hours in triplicate. For each experiment, 500 ml of wine were placed in closed graduated cylinders, at room temperature (20ºC, in the dark). After 48 hours of adding the fining agents and oenological additives, a centrifugation step at 2500 rpm for 10 min allowed separating sediment from wine for further analyses. All fining agents were prepared according to the manufacturer‟s recommendations. The recommended minimum and maximum concentrations for all fining agents were used respectively as concentration 1 and 3. The concentration 2 was the mean concentration of the two others. Untreated wine was used as control. The specific concentrations of compounds used are given in Table IV.1.

Table IV.1: The concentration of enological agents employed in this study

Agents	Control	Conc. 1	Conc. 2	Conc. 3
Egg albumin (EA)	0	5 g/hl	10 g/hl	15 g/hl
PvPP + Casein (PvPP + Cas)	0	15 g/hl	52.5 g/hl	90 g/hl
Bentonite (B)	0	10 g/hl	45 g/hl	80 g/hl
Vegetable protein (VP)	0	1 g/hl	3 g/hl	5 g/hl
Gelatin (G)	0	4 cl/hl	7 cl/hl	10 cl/hl
Tannins (T)	0	10 g/hl	25 g/hl	40 g/hl
Mannoproteins (M)	0	10 g/hl	25 g/hl	40 g/hl

IV.2.3. SPECTROPHOTOMETRIC ANALYSIS OF POLYPHENOLS (see II.1.2.5. p. 88)

IV.2.4. HPLC ANALYSIS OF PHENOLIC COMPOUNDS (see II.1.2.6. p. 89)

IV.2.5. STATISTICAL DATA TREATMENT

All experiments were carried out in triplicate. Analysis of variance (ANOVA) and Tukey‟s honestly significant difference (HSD) test were used for mean separation, with a significant level of 95% ($p < 0.05$).These statistical analyses, together with PCA, were conducted using Xlstat software (2014).

IV.3. Results and discussion

IV.3.1. SPECTROSCOPIC ANALYSES

IV.3.1.1. Chromatic parameters and Antioxidant activity

Table IV.2 shows the chromatic properties and the antioxidant activity of wines. The addition of fining agents and oenological additives decreased the color intensity and increased the hue values of most of the treated wines compared to the control one. The high concentration of bentonite had the highest impact on the color of wines by decreasing the intensity. Decreases in color intensity (0 to – 5%), were accompanied by increases of hue (+ 1.9% to 2.68%) in the wines clarified by this fining agent. So the bentonite affected ionized anthocyanins decreasing in this way the intensity of red color and consequently influences the hue of the wine (Stankovic et al., 2004).

Fining with PvPP + casein showed an equal importance to that of bentonite for the decreasing in color intensity (-1.56 to - 4.30%), due to the effect of mixture of fining agents. Vegetable proteins had the less impact on color intensity comparing to the control. These observations are in accordance with those obtained by Gonzalez-Neves et al. (2014). They found that bentonite affected the most the color intensity while the plant proteins did not affect significantly the color intensity. The difference in behavior between the used agents for the same type of wine determines a wide diversity of molecular masses, isoelectric points and surface charge densities that modify strongly their interactions with polyphenols and their effect on the color of wines (Marchal et al., 2002; Maury et al., 2003).

The total polyphenol index (TPI) is hugely affected by the fining treatments. The decrease of TPI is explained by the remove of some classes of polyphenols by the fining treatments especially by bentonite. The addition of tannins especially at high concentration leads to a significant increase in TPI compared to the control.

The antioxidant activity of wines was evaluated by the ABTS assay which is a simple and efficient method for the evaluation of antiradical activity. The results were expressed as Gallic acid equivalent (mg/ml of wine). A little decrease in the antioxidant activity is observed when the wines are treated with fining agents comparing to control except for tannins. When tannins are added an increase in antioxidant activity is observed but it is independent from the concentration. It seems that the type of added tannins influence more the antioxidant activity than the concentration.

Table IV.2: The total polyphenol index, chromatic parameters (CI and Hue), and antioxidant activity of control and treated wines.

Agents concentrations	Treatments	TPI	CI	Hue	ABTS mg/ml (GAE)
Concentration 1	C	84.60 ± 2.62^{a}	2.93 ± 0.01^{a}	0.72 ± 0.01^{a}	2.91 ± 0.06^{b}
	EA	75.07 ± 0.46^{ab}	2.89 ± 0.03^{bcd}	0.73 ± 0.001^{a}	2.90 ± 0.00^{b}
	PvPP+ Cas	80.97± 4.8^{a}	2.88 ± 0.03^{bcd}	0.74 ± 0.02^{a}	2.95 ± 0.00^{a}
	B	81.70 ± 2.35^{a}	2.95 ± 0.03^{a}	0.74 ± 0.00^{a}	2.91 ± 0.03^{b}
	VP	76.33 ± 0.84^{ab}	2.91 ± 0.006^{abc}	0.72 ±0.00^{a}	2.91 ± 0.08^{b}
	G	75.73 ± 1.81^{ab}	2.93 ± 0.012^{a}	0.72 ± 0.00^{a}	2.91 ± 0.00^{b}
	T	86.67 ± 2.96^{a}	2.91 ± 0.02^{abc}	0.73 ± 0.00^{a}	1.40 ± 0.00^{c}
	M	84.47 ± 3.17^{a}	2.88 ± 0.005^{cd}	0.73 ± 0.00^{a}	2.97 ± 0.06^{a}
Concentration 2	C	84.6 ± 2.62^{a}	2.93 ± 0.005^{b}	0.72 ± 0.01^{b}	2.91 ± 0.06^{cd}
	EA	74.13 ± 1.88^{b}	2.89 ± 0.002^{c}	0.73 ± 0.00^{ab}	2.90 ± 0.06^{d}
	PvPP+ Cas	77.43 ± 1.87^{b}	2.85 ± 0.005^{d}	0.73 ± 0.00^{ab}	3.10 ± 0.00^{b}
	B	78.53 ± 1.75^{ab}	2.88 ± 0.007^{c}	0.74 ± 0.00^{a}	2.90 ± 0.03^{d}
	VP	82.43 ± 1.82^{a}	2.93 ± 0.02^{b}	0.73 ± 0.00^{b}	2.90 ± 0.09^{d}
	G	80.63 ± 1.04^{a}	2.99 ± 0.06^{a}	0.74 ± 0.01^{a}	2.92 ± 0.00^{c}
	T	87.33 ± 2.28^{a}	2.88 ± 0.00^{c}	0.73 ± 0.01^{ab}	1.30 ± 0.00^{e}
	M	84.17 ± 1.99^{a}	2.89 ± 0.01^{c}	0.73 ± 0.00^{ab}	3.32 ± 0.03^{a}
Concentration 3	C	84.60 ± 2.62^{ab}	2.93 ± 0.01^{ab}	0.72 ± 0.01^{cd}	2.91 ± 0.06^{de}
	EA	74.77 ± 0.32^{b}	2.89 ± 0.01^{d}	0.73 ± 0.00^{bcd}	2.95 ± 0.00^{c}
	PvPP+ Cas	78.33 ± 1.86^{b}	2.81 ± 0.00^{e}	0.73 ± 0.00^{bc}	3.30 ± 0.1^{b}
	B	74.17 ± 1.19^{b}	2.78 ± 0.00^{e}	0.75 ± 0.00^{a}	2.92 ± 0.05^{de}
	VP	78.90 ± 2.94^{b}	2.95 ± 0.02^{a}	0.73 ± 0.00^{bc}	2.90 ± 0.00^{e}
	G	80.83 ± 2.17^{b}	2.93 ± 0.01^{abc}	0.73 ± 0.01^{bc}	2.93 ± 0.08^{d}
	T	94.83 ± 0.64^{a}	2.91 ± 0.01^{bcd}	0.73 ± 0.00^{bc}	1.35 ± 0.00^{f}
	M	86.00 ± 1.63^{ab}	2.91 ± 0.03^{cd}	0.74 ± 0.01^{a}	3.33 ± 0.03^{a}

Mean value ± standard deviation. Different letters within the same row represents significant differences according to Tukey HSD test ($p < 0.05$).

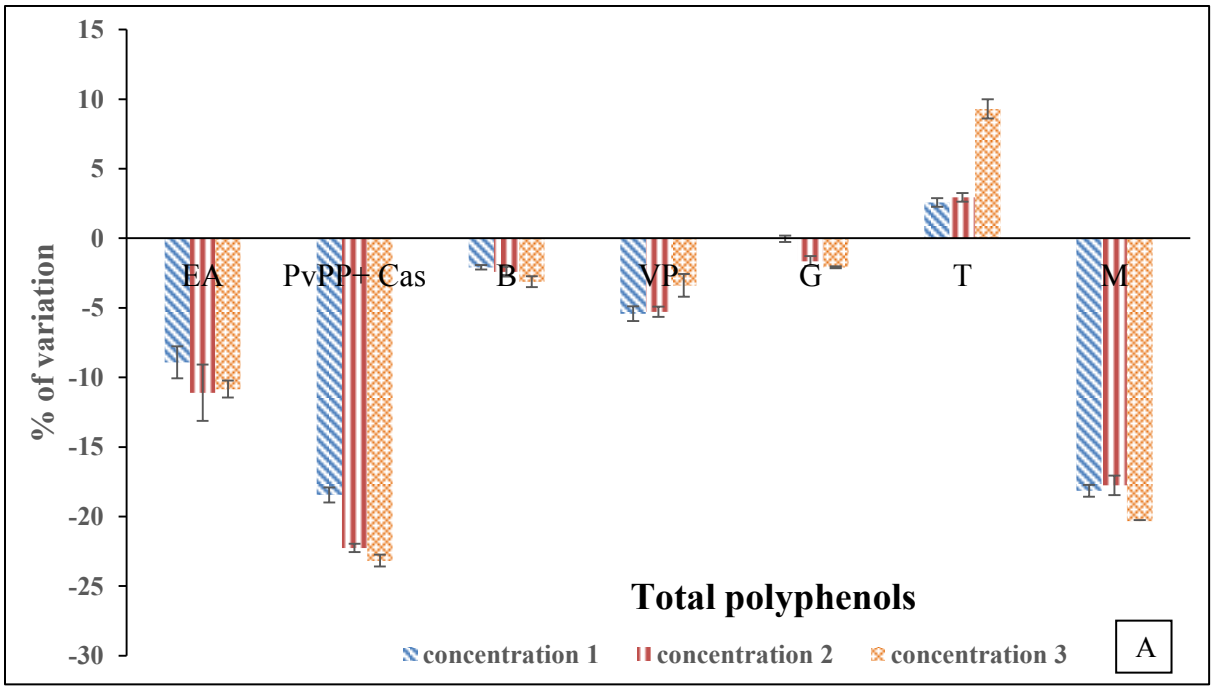
The correlation between the antioxidant activity and the total polyphenol has been justified by several authors (Di Majo et al., 2008; Ertan-Anli and Vural, 2009; Galmarini et al., 2013). Majo et al. (2008) showed a linear correlation between antioxidant capacity and the content of total polyphenols. In our case, it seems that the antiradical activity is due to the flavan-3-ol fraction more than the anthocyanins because when observing the treatment with bentonite, which decreases hugely the anthocyanins contents, no decreases in antioxidant activity is observed.

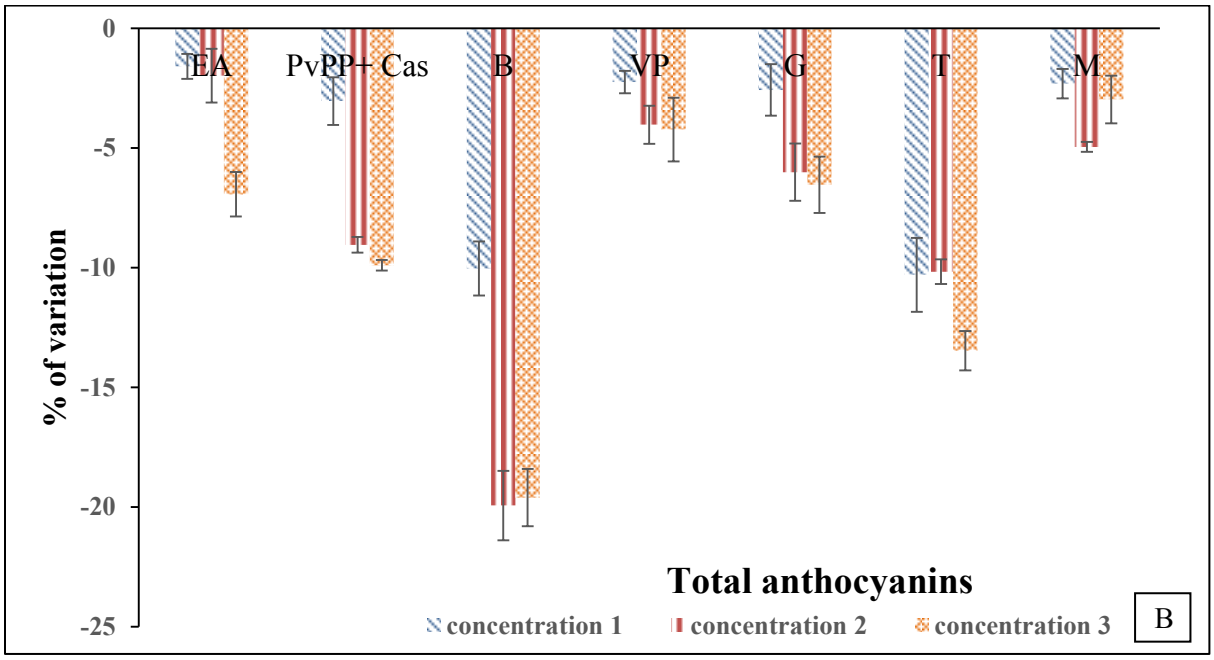
IV.3.1.2. Total polyphenols, and total anthocyanins and total tannins

After fining, total polyphenols (Fig. IV.1-A), total anthocyanins (Figure IV.1-B) and total tannins (Figure IV.1-C) of the wines were compared with those registered before treatments (the control). All treated wines showed a decrease in the content of total polyphenol except wines added by exogenous tannins; even though it is not significant except that for the maximum concentration (concentration 3). These results are due principally to the effect of different agents on anthocyanins (Figure IV.1-B) and tannins (Figure IV.1-C) contents of wines. PvPP + casein had the most important effect, with decreases of total polyphenols levels between 17.34% (15 g/hl) and 23.16% (90 g/hl) and total tannins around 7%. PvPP is a synthetic polymer that complexes with wine phenolic compounds by hydrogen bonds. Han et al. (2015) demonstrated that wines made from Cabernet Sauvignon cultivar treated with PvPP showed significant losses in polyphenol concentration as PvPP binds and removes phenolics. In addition to PvPP, casein fining can promote a decrease in polyphenol in monomeric and oligomeric flavanols as well as proanthocyanidins as shown by Braga et al. (2007).

Mannoproteins was the second agent that causes reduction of total polyphenols (20%) and total tannins (6%) contents when high concentrations are used. These results are in accordance with those obtained by (Guadalupe and Ayestaran, 2008) who showed that mannoproteins addition to wines coincided with substantial reduction in proanthocyanidin and pigments. They suggested a precipitation of the co-aggregates mannoproteins-tannins and mannoproteins-pigments. In contrary, Rodrigues et al. (2012) showed that the addition of commercial mannoproteins to red wine did not have a significant effect on color and tannins while compared to untreated wine. The only effect shown in this study is a delay of tannins polymerization in red wines. Nguela et al. (2016) showed interactions between mannoproteins and wine tannins which led to stable colloidal aggregates with finite size. This was attributed to the glycosyl moiety of mannoproteins which

may prevent multiple bridging between tannins and their protein part or may form a hydrophilic and negatively charged shell around aggregates that stop their growth. The remaining fining agents as bentonite, gelatin, egg albumin and vegetable proteins showed less effect on total polyphenol and total tannins contents.





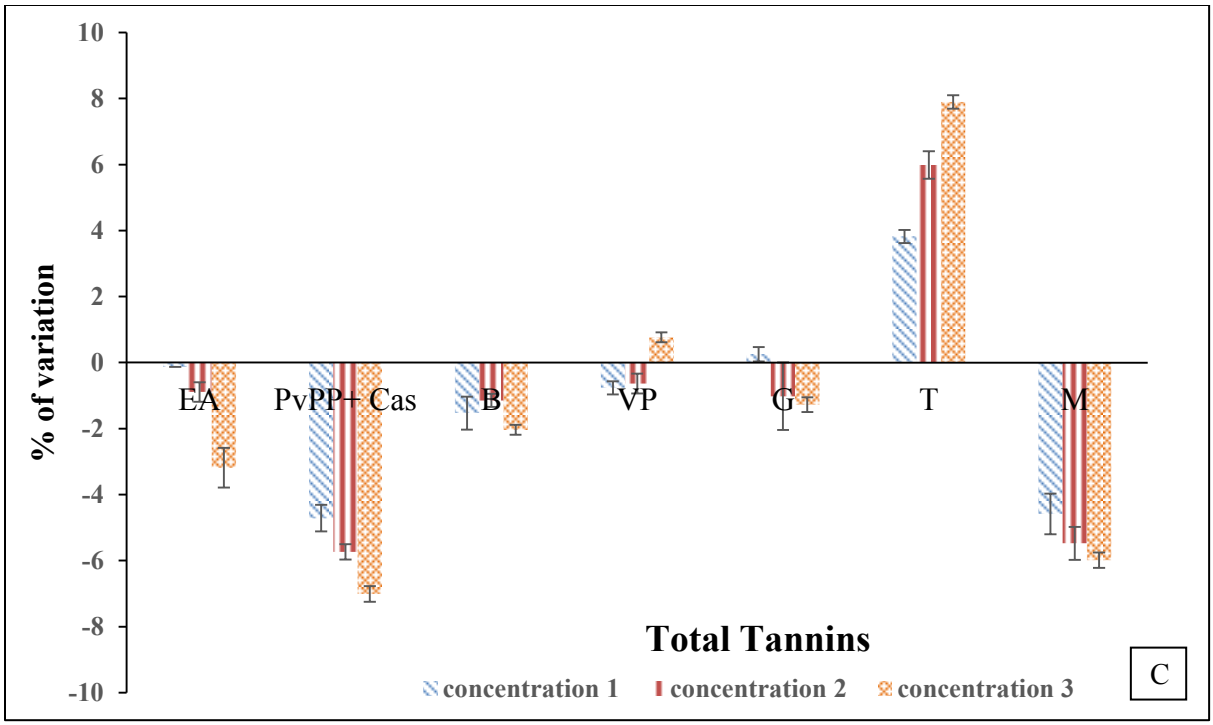


Figure IV.1: The variation of total polyphenol (A), total anthocyanins (B) and total tannins (C) after treatment of wines with fining agents. Amounts of phenolic compounds were compared to wines before treatment (control) as external reference (0% of variation)

Bentonite had the highest impact on the anthocyanins contents of wines. The concentration of bentonite has an important impact on the decrease of anthocyanins levels. Decreases of the levels of anthocyanins by bentonite, which is particularly emphasized with a dose of 80 g/hl, were comprises among 10% and 19.6% in relation to their concentration in control wines. These proportions are less than the results reported by Stankovic et al. (2012) and González-Neves et al. (2014) with other grape varieties, who found that the use of bentonite significantly decreased the anthocyanin levels between 9.8% and 35%. The different behavior found in our study must relate to the wine age. The highest decrease in anthocyanins contents by bentonite were verified in older wines, so the impact of bentonite on the colloidal matter could explain the results (Ribéreau Gayon et al., 2006). Bentonite is mainly negatively-charged clay of volcanic origin which indirectly binds phenols that have complexed with proteins and can also bind anthocyanins, with a resulting loss of color (Donovan et al., 1999). As cation exchanger clay, bentonite can remove other positively charged molecules as anthocyanins (Chagas et al., 2012).

The addition of oenological tannins exhibit antagonist effects. The addition increases the total polyphenol by 9% at higher concentration and total tannins by 8% at higher concentration while it decreases significantly the total anthocyanins. The oenological tannins are the second agent after the bentonite to lower the content of total anthocyanins between 10.29% and 13.46%. Several tannin products can be found on the market with different origins and chemical composition. The oenological tannins used in this study are condensed tannins. Condensed tannins can combine with anthocyanins and generate colorless compounds and stabilize wine color. This can explain the decrease in anthocyanins contents. Bautista-Ortin et al. (2005) showed that the addition of 400 mg/l of condensed tannins did not influence the anthocyanins content of Monastrell wines compared to the control. The same observations were made by Parker et al. (2007) while testing the addition of tannins at either prefermentation or postfermentation level. Harbertson et al. (2012) studied the impact of adding of exogenous tannins at different concentrations on wine polyphenol content. They showed that the addition with the recommended concentrations had a little impact on wine polyphenol. The addition of tannins was found to retard the degradation of most anthocyanins in the process of winemaking (Liu et al., 2013).

IV.3.2. DETERMINATION OF POLYPHENOL CLASSES BY RP-HPLC

The individual anthocyanin composition of untreated and treated wines is represented in table IV.3. In the control wine, malvidin-3-glucoside was the major individual anthocyanin followed by delphinidin-3-glucoside, peonidin-3-glucoside and cyanidin-3-glucoside. The petunidin-3-glucoside is not detected in the Cabernet Sauvignon wine used for this study. The levels of anthocyanin monomers composition were slightly diminished by most of the treatments except mannoproteins (Table IV.3). Although bentonite showed the highest decrease in total anthocyanins (Figure IV.1-B), this latter minimally correlated with the loss of glycosylated anthocyanins (Table IV.3), which suggests that bentonite eliminated other compounds of anthocyanins based on acetyl and coumaroyl-glycosides. Results showed that the treatment with commercial mannoproteins can lead to a significant increase in monomeric anthocyanins especially malvidin-3-glucoside comparing to the control. In 2012, Del Barrio-Galan et al. observed the same tendency when studying the effect of different commercial mannoproteins on the phenolics of red wine. They showed that 2 of the tested commercial mannoproteins increase the concentrations of monomeric anthocyanins. In fact, mannoproteins favored the formation of

new anthocyanins pigments which are more stable and resistant to pH changes and oxidation reactions.

Table IV.3: Monomeric anthocyanins of control and treated wines

Agents concentrations	Treatments	Delphinidin-3-glc (mg/l)	Cyanidin -3-glc (mg/l)	Peonidin-3-glc (mg/l)	Malvidin-3-glc (mg/l)	Σglycosylated anthocyanins
Conc. 1	C	24.96 ± 0.79^{c}	8.31 ± 0.11^{a}	9.41 ± 0.12^{a}	243.14 ± 2.66^{d}	285.82 ± 3.68^{d}
	EA	25.26 ± 0.03^{bc}	5.47 ± 0.12^{cd}	5.77 ± 0.14^{c}	220.35 ± 1.37^{e}	256.85 ± 1.66^{e}
	PvPP+ Cas	27.45 ± 0.34^{a}	5.96 ± 0.41^{bc}	7.66 ± 0.26^{b}	288.27 ± 0.48^{b}	329.34 ± 1.49^{b}
	B	27.42 ± 0.21^{a}	5.21 ± 0.09^{d}	6.89 ± 0.13^{bc}	248.27 ± 6.48^{c}	287.79 ± 6.91^{c}
	VP	26.71 ± 0.75^{ab}	5.18 ± 0.26^{d}	5.84 ± 0.15^{c}	223.22 ± 1.48^{e}	260.95 ± 2.64^{e}
	G	25.39 ± 0.26^{bc}	5.38 ± 0.13^{cd}	5.33 ± 0.05^{c}	219.64 ± 3.00^{f}	255.74 ± 3.44^{f}
	T	25.68 ± 0.50^{bc}	6.40 ± 0.93^{bc}	6.42 ± 0.57^{bc}	224.90 ± 3.72^{e}	263.40 ± 5.72^{e}
	M	26.17 ± 0.68^{abc}	6.82 ± 0.12^{b}	7.84 ± 0.29^{ab}	315.86 ± 5.02^{a}	356.69 ± 6.11^{a}
Conc. 2	C	24.96 ± 0.79^{c}	8.31 ± 0.11^{a}	9.41 ± 0.12^{a}	243.14 ± 2.66^{bc}	285.82 ± 3.68^{b}
	EA	25.01 ± 0.36^{c}	5.51 ± 0.26^{b}	5.80 ± 0.26^{c}	222.28 ± 2.11^{d}	258.68 ± 2.99^{e}
	PvPP+ Cas	26.53 ± 0.10^{b}	5.36 ± 0.45^{b}	6.42 ± 0.28^{c}	251.10 ± 0.68^{b}	289.41 ± 1.51^{b}
	B	25.18 ± 0.15^{c}	4.98 ± 0.52^{b}	6.88 ± 1.41^{bc}	236.75 ± 2.44^{c}	273.79 ± 4.52^{c}
	VP	27.17 ± 0.73^{ab}	5.30 ± 0.25^{b}	6.02 ± 0.18^{c}	223.30 ± 0.90^{d}	261.97 ± 2.06^{d}
	G	27.88 ± 0.27^{a}	6.07 ± 1.21^{b}	5.71 ± 0.32^{c}	223.46 ± 2.53^{d}	263.12 ± 4.33^{d}
	T	25.33 ± 0.16^{c}	5.76 ± 0.10^{b}	5.78 ± 0.25^{c}	226.10 ± 2.30^{d}	262.97 ± 2.81^{d}
	M	26.83 ± 0.01^{ab}	5.44 ± 0.37^{b}	8.30 ± 0.17^{ab}	325.09 ± 4.29^{a}	365.66 ± 4.84^{a}
Conc. 3	C	24.96 ± 0.79^{d}	8.31 ± 0.11^{a}	9.41 ± 0.12^{a}	243.14 ± 2.66^{b}	285.82 ± 3.68^{b}
	EA	26.16 ± 0.66^{d}	5.26 ± 0.15^{cd}	5.53 ± 0.23^{e}	225.64 ± 0.74^{c}	262.59 ± 1.78^{d}
	PvPP+ Cas	25.43 ± 0.36^{d}	4.91 ± 0.13^{d}	6.62 ± 0.26^{c}	246.03 ± 0.57^{b}	282.99 ± 1.32^{b}
	B	27.80 ± 0.63^{bc}	5.14 ± 0.14^{cd}	6.38 ± 0.51^{cd}	224.74 ± 3.11^{b}	264.06 ± 4.39^{b}
	VP	27.85 ± 0.45^{b}	5.36 ± 0.27^{cd}	6.04 ± 0.21^{cde}	226.68 ± 1.84^{c}	265.93 ± 2.77^{c}
	G	26.34 ± 0.25^{bcd}	5.08 ± 0.11^{cd}	5.93 ± 0.17^{cde}	226.78 ± 2.61^{c}	264.13 ± 3.14^{c}
	T	26.10 ± 0.44^{cd}	5.57 ± 0.40^{c}	5.86 ± 0.15^{de}	228.25 ± 5.43^{c}	265.78 ± 6.42^{c}
	M	30.24 ± 0.97^{a}	6.40 ± 0.28^{b}	8.59 ± 0.13^{b}	338.15 ± 1.30^{a}	383.38 ± 2.68^{a}

Mean value ± standard deviation. Different letters within the same row represents significant differences according to Tukey HSD test ($p < 0.05$).

Table IV.4: The monomeric and dimeric flavan-3-ols, phenolic acids and resveratrol of control and treated wines

		Flavan-3-ols						Phenolic acids			Stilbenes
Agents concentrations	Treatments	Catechin	Epicatechin	Epigallo-catechin	Epicatechin gallate	Procyanidin B1	Procyanidin B2	Gallic acid	Caffeic acid	Ferulic acid	Resveratrol
Conc. 1	C	68.41 ± 0.38^{a}	121.24 ± 0.56^{a}	300.71 ± 3.73^{a}	41.05 ± 1.30^{a}	87.61 ± 1.47^{a}	139.98 ± 3.08^{a}	41.21 ± 0.54^{a}	2.06 ± 0.02^{a}	30.95 ± 0.72^{a}	3.87 ± 0.01^{a}
	EA	68.41 ± 0.78^{a}	122.80 ± 2.27^{a}	301.06 ± 1.08^{a}	41.36 ± 2.27^{a}	86.34 ± 2.23^{a}	108.17 ± 1.53^{c}	41.34 ± 0.55^{a}	2.05 ± 0.01^{a}	31.09 ± 1.58^{a}	3.95 ± 0.14^{a}
	PvPP+ Cas	70.31 ± 1.15^{a}	121.66 ± 3.14^{a}	306.68± 5.57^{a}	42.58 ± 0.48^{a}	88.25 ± 1.70^{a}	115.51 ± 2.94^{bc}	41.73 ± 0.07 ^{a}	2.05 ± 0.01^{a}	31.27 ± 1.43^{a}	3.85 ± 0.03^{a}
	B	67.46 ± 3.59^{a}	115.54 ± 12.57^{a}	184.24 ± 9.18^{b}	41.22 ± 0.66^{a}	77.43 ± 0.40^{b}	123.55 ± 2.18^{b}	41.19 ± 0.54^{a}	2.04 ± 0.01^{a}	31.22 ± 0.84^{a}	3.92 ± 0.05^{a}
	VP	68.39 ± 1.13^{ab}	123.30 ± 2.52^{a}	302.86 ± 4.78^{a}	44.19 ± 2.58^{a}	85.71 ± 0.99^{ab}	111.34 ± 1.36^{c}	41.31 ± 0.59 ^{a}	2.05 ± 0.01^{a}	30.88 ± 1.79^{a}	3.99 ± 0.05^{a}
	G	67.68 ± 2.38^{a}	113.24 ± 3.16^{a}	294.97± 12.36^{a}	39.39 ± 1.37^{a}	87.42 ± 3.70^{a}	137.81 ± 1.14^{a}	41.74 ± 0.04^{a}	2.05 ± 0.01^{a}	30.34 ± 0.69^{a}	3.86 ± 0.05^{a}
	T	69.86 ± 0.94^{a}	121.02 ± 5.27^{a}	302.50 ± 9.30^{a}	40.35 ± 2.55^{a}	91.58 ± 2.43^{a}	92.20± 8.26^{d}	41.33 ± 0.56^{a}	2.05 ± 0.01^{a}	31.42 ± 1.31^{a}	3.88 ± 0.04^{a}
	M	69.63 ± 1.41^{a}	123.56 ± 3.6^{a}	301.25 ± 1.44^{a}	43.39 ± 2.69^{a}	86.54 ± 6.56^{a}	95.46 ± 1.71^{d}	41.57± 0.02^{a}	2.06 ± 0.01^{a}	31.05 ± 0.78^{a}	3.92 ± 0.04^{a}
Conc. 2	C	68.42 ± 0.38^{ab}	121.24± 0.56^{a}	300.71 ± 3.73^{a}	41.05 ± 1.30^{a}	87.61 ± 1.47^{ab}	139.98 ± 3.08^{a}	41.21± 0.54^{a}	2.06 ± 0.02^{a}	30.95 ± 0.72^{a}	3.87 ± 0.01^{a}
	EA	69.10 ± 1.30^{ab}	123.55 ± 5.38^{a}	301.66 ± 5.68^{a}	40.90 ± 0.40^{a}	85.52 ± 3.05^{ab}	110.03 ± 3.84^{b}	40.99 ± 0.60^{a}	2.05 ± 0.01^{a}	30.87 ± 1.12^{a}	3.93 ± 0.16^{a}
	PvPP+ Cas	67.42 ± 1.65^{b}	118.14 ± 5.02^{a}	284.28 ± 0.89^{b}	44.55 ± 3.16^{a}	81.39 ± 3.62^{b}	110.20 ± 3.80^{b}	41.59 ± 1.00 ^{a}	2.05 ± 0.01^{a}	31.76 ± 0.36^{a}	3.83 ± 0.02^{a}
	B	69.82 ± 3.01^{ab}	123.91 ± 5.10^{a}	179.59 ± 5.92^{c}	44.81 ± 2.64^{a}	86.19 ± 2.14^{ab}	112.46 ± 1.19^{b}	41.59 ± 0.01^{a}	2.05 ± 0.01^{a}	31.26 ± 1.51^{a}	3.84 ± 0.09^{a}
	VP	69.37 ± 1.06^{ab}	124.30 ± 3.33^{a}	307.57± 4.41^{a}	44.42 ± 2.83^{a}	87.34 ± 2.28^{ab}	112.19 ± 1.81^{b}	41.61 ± 0.05^{a}	2.05 ± 0.01^{a}	30.88 ± 1.92^{a}	3.86 ± 0.05^{a}
	G	68.86 ± 0.58^{ab}	122.58 ± 0.92^{a}	297.69 ± 3.69^{a}	39.37 ± 1.64^{a}	85.86 ± 0.62^{ab}	108.01 ± 2.99^{b}	41.65 ± 0.07^{a}	2.06 ± 0.01^{a}	31.11 ± 0.89^{a}	3.85± 0.05^{a}
	T	72.41 ± 2.40^{a}	124.48 ± 5.91^{a}	296.71 ± 4.22^{ab}	42.31 ± 2.74^{a}	89.45 ± 2.33^{a}	100.71 ± 6.74^{b}	41.65± 0.04^{a}	2.05 ± 0.01^{a}	30.55 ± 1.25^{a}	3.79 ± 0.03^{a}
	M	70.04 ± 1.29^{ab}	123.49 ± 3.64^{a}	298.18 ± 6.95^{a}	43.72 ± 2.93^{a}	88.93 ± 2.35^{a}	105.24 ± 7.31^{b}	40.74 ± 0.64^{a}	2.05 ± 0.01^{a}	30.87 ± 1.98^{a}	3.83 ± 0.06^{a}
Conc. 3	C	68.41 ± 0.38^{b}	121.24 ± 0.56^{a}	300.71 ± 3.73^{a}	41.05 ± 1.30^{a}	87.61 ± 1.50^{a}	139.98 ± 3.08^{a}	41.21 ± 0.54^{a}	2.05 ± 0.02^{a}	30.95 ± 0.73^{a}	3.87 ± 0.01^{a}
	EA	68.27 ± 5.75^{b}	120.61 ± 7.64^{a}	295.93 ± 3.73^{ab}	39.94 ± 0.64^{a}	79.71 ± 1.00^{b}	105.35± 0.84^{c}	41.93 ± 0.65^{a}	2.05 ± 0.01^{a}	30.52 ± 1.63^{a}	3.96 ± 0.14^{a}
	PvPP+ Cas	67.18 ± 0.72^{b}	119.49 ± 0.99^{a}	289.44 ± 1.76^{b}	41.12 ± 0.63^{a}	83.44 ± 1.01^{ab}	112.95 ± 2.63^{bc}	41.7 ± 0.05^{a}	2.05 ± 0.01^{a}	31.23 ± 1.18^{a}	3.93 ± 0.03^{a}
	B	69.65 ± 0.69^{b}	124.13 ± 2.55^{a}	177.24 ± 2.73^{c}	43.23 ± 1.48^{a}	86.18 ± 1.51^{a}	115.87 ± 2.85^{b}	41.66 ± 0.03^{a}	2.05 ± 0.02^{a}	31.13 ± 1.74^{a}	3.81 ± 0.06^{a}
	VP	68.26 ± 0.97^{b}	122.69 ± 4.1^{a}	304.13 ± 7.04^{a}	44.21 ± 3.43^{a}	84.74 ± 3.89^{ab}	110.85 ± 2.78^{bc}	41.65 ± 0.03^{a}	2.05 ± 0.01^{a}	31.07 ± 1.49^{a}	3.85 ± 0.04^{a}
	G	68.29 ± 1.15^{b}	120.39 ± 2.95^{a}	296.45 ± 3.88^{ab}	39.92 ± 0.01^{a}	84.16 ± 2.79^{ab}	107.92 ± 0.41^{c}	41.64± 0.01^{a}	2.05 ± 0.01^{a}	30.71 ± 1.48^{a}	3.87 ± 0.02^{a}
	T	76.91 ± 1.06^{a}	129.99 ± 3.70^{a}	304.33± 6.61^{a}	45.24 ± 2.90^{a}	89.53 ± 0.22^{a}	110.4 ± 3.23^{bc}	41.33 ± 0.57^{a}	2.05 ± 0.01^{a}	31.22 ± 1.28^{a}	3.89 ± 0.01^{a}
	M	70.15 ± 1.14^{b}	125.08 ± 4.22^{a}	302.01 ± 7.05^{a}	45.07 ± 2.83^{a}	86.59 ± 3.12^{a}	112.05 ± 3.9^{bc}	41.55 ± 0.22^{a}	2.05 ± 0.04^{a}	30.84 ± 2.05^{a}	3.85 ± 0.06^{a}

Mean value ± standard deviation. Different letters within the same row represents significant differences according to Tukey HSD test ($p < 0.05$)

Table IV.4 represents the concentration of monomeric and dimeric flavanols as well as some phenolic acids and resveratrol. Monomeric flavanols were little affected by the fining agents except epigallocatechin. Epigallocatechin was the principal phenolic removed by bentonite fining agent (decreases of 41% by the maximum recommended concentration). Also, bentonite decreased significantly the concentrations of dimeric flavanols (procyanidin B1 and procyanidin B_2). Bentonite may indirectly binds phenols that have complexed with proteins (Donavan et al., 1999).

PVPP + casein showed to mainly remove catechin and epigallocatechin. Actually PVPP is a synthetic polymer that complexes with phenolic wine components by hydrogen bond formation. It has an affinity for low molecular weight phenols (catechin) and for compounds with a higher degree of hydroxylation (epigallocatechin, with three hydroxyl radicals) (MCMur-rought et al., 1995).

The mainly flavanols removed by gelatin and egg albumin were procyanidin B_1 and B_2. Procyanidin B_2 was decreased by 24.71%, followed by procyanidin B_1 (11.09%) for egg albumin while gelatin scored a decrease of 22.9% and 4% respectively. These results are in good agreement with the finding of Oberholster et al. (2013), who showed that both egg albumin and gelatin significantly decreased the mean degree of polymerization (mDP) of the wine tannins by respectively 26.4% and 25.20%. Also, our results are in agreement with the findings of other researchers (Cosme et al., 2009; Maury et al., 2003; Sarni-Manchado et al., 1999).

Vegetable proteins decreased procyanidin B_2 by 20.80% as efficiently as gelatin (22.90%). These results are in accordance with those obtained by Jauregi et al. (2016) who showed that whey proteins reduced astringency in wine as efficiently as gelatin, mainly via hydrophobic interactions and hydrogen bonding with tannins leading to their aggregation and precipitation. Other authors (González-Neves et al., 2014) showed that fining with vegetable proteins had no significant effect on proanthocyanidins contents of wines. Indeed, there is a wide variety of commercial preparations; the evaluation of it is use must refer to the characteristics of each particular product (Marchal et al., 2002; Tschiersch et al., 2010). The protein fining agents were found to bind more easily with condensed tannins more than monomeric tannins (Sarni-Manchado et al., 1999).

The addition of mannoproteins did not affect the monomeric flavanols as others author showed (Guadalupe and Ayestarán, 2008). Procyanidin B_2 was the only flavanols decreased (-24.82%). Previous studies performed also observed an interaction of mannoproteins with procyanidins (Rodrigues et al., 2012; Guadalupe and ayestarán, 2008).

The addition of tannins was shown to increase total polyphenols levels and total tannins levels. No significant effect was observed on the monomeric flavanols because the added tannins are condensed tannins which cannot release monomeric flavanols. Surprisingly, the addition of condensed tannins decreases the levels of procyanidin B_2 (-34.1%). This can be explained by the polymerization between added tannins and procyanidin B_2. The self-association of flavanols and their aggregation have been demonstrated in the literature (Pianet et al., 2008). It was demonstrated that the hydrophobic interactions are the major driving forces to the flavanols self-association.

All wine treatments didn't show any effect on the phenolic acids and resveratrol contents in the wines. This is suggesting there is no interaction between small phenolic compounds and macromolecules or particles.

IV.3.3. EFFECT OF TREATMENT CONCENTRATIONS ON THE PHENOLIC COMPOSITION OF WINES

In order to examine the effect of different agents concentrations on the phenolic composition of wines, principal component analysis was applied to a matrix of four variables (anthocyanins, total polyphenols, tannins and ABTS) explained by the first two principal components (PC1 and PC2) and representing 88.10% of the total variance (Figure IV.2). Evaluating the positions of fining agents at different concentrations 5 groups were formed. The first group was formed by egg albumin and mannoproteins, situated in the left upper part of the coordinate, which is opposite to total polyphenols, tannins and ABTS (relative to PC1, with the same direction of anthocyanins (relative to PC2). The second group was composed by control, vegetable protein and gelatin, located in the right upper part of the coordinate, positively correlated with total polyphenols and tannins and opposite to anthocyanins and ABTS. The third group included tannins located in the upper right part of the coordinate which was fitted with total polyphenols and tannins. The fourth group was constituted by bentonite situated in the right lower part of the coordinate opposite to anthocyanins and ABTS. The last one involved PvPP + casein located in the left lower part of the coordinate opposite to total polyphenols, tannins and anthocyanins. The best combination that fit the four variables without excess removing of different groups of phenolic compounds was the second group, confirming that vegetable protein and gelatin fining agents had minimal effect on the phenolic composition of wines. The results of PCA showed the importance of using the recommended minimum amount of all fining agents for high phenolic compounds and antioxidant activity.

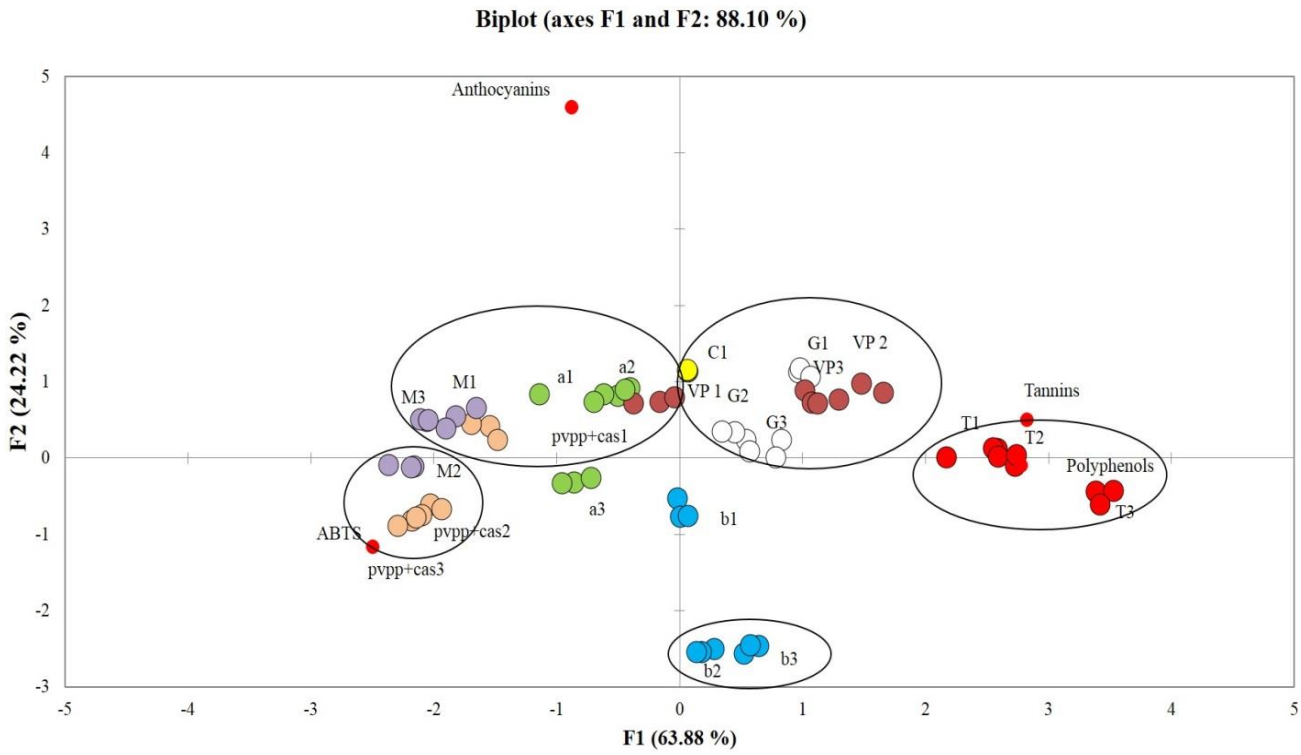


Figure IV.2. PCA Biplot of the two first principal components of analysed parameters: Anthocyanins (mg/l), total polyphenols (mg/l GAE), ABTS (mg/l GAE) and Tannins (mg/l) in samples treated with different fining agent (C, control; EA, egg albumin; PvPP + Cas, polyvinylpyrrolidone + Casein; B, bentonite; VP, vegetable proteins; G, gelatin; T, tannins; M, mannoproteins) at different concentrations (1, concentration 1; 2, concentration 2; 3, concentration 3)

IV.4. Conclusion

Using fining agents, adding tannins and commercial mannoproteins for red wines must be taken with care, since these agents determined a different impact on the organoleptic characteristic of wines according to their nature, the applied dose and the style of wine. The most remarkable effects were those obtained by bentonite which had negative impact on the anthocyanins contents and wine color, in addition mannoprotein and PvPP + casein decreased significantly tannin levels, while vegetable protein and gelatin revealed the less impact on the wine phenolic composition. Antioxidant activity was positively affected by the addition of condensed tannins. After all, the results of principle components analyses showed the importance of a low concentration of fining agents for high antioxidant activity and high phenolic compounds.

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Conclusions and Perspectives

The general objective of this work was to assess the impact of the winemaking process on the composition and biological activities of Lebanese wines, since these wines have been little studied so far. The purpose was to evaluate in particular the impact of maceration time (0, 2, 4, 8, 24 and 48h) and temperatures with or without added enzymes (10, 60, 70, 80, 25°C, 70°C + enzymes), effect of two different commercial yeast strains (X and Y) during alcoholic fermentation, effect of terroirs and vintages, impact of five different oenological fining practices (egg albumin, PVPP + casein, bentonite, gelatin and vegetable proteins) and two oenological additives (tannins and mannoproteins); as well as the effect of different fining concentrations on the chromatic characteristics, phenolic composition, and biological activities of must and wines of two grape varieties (Cabernet Sauvignon and Syrah) from two distinct Lebanese regions (Saint Thomas and Florentine) during two consecutive years (2014 and 2015).

Concerning the maceration step, results showed that the pre-fermentation heat treatment of grapes is more efficient for the extraction of polyphenols than the cold maceration and the traditional maceration during alcoholic fermentation. The pre-fermentation cold maceration didn‟t show big evolution in the extraction kinetics of phenolic compounds during 48 hours. Analysis of wine samples revealed a systematic increase in the concentration of tannins with temperature and over time. High temperatures favored also anthocyanin extraction but a degradation of these compounds was observed when the maceration is extended beyond 8 hours. Also, high temperatures favored the extraction of total polyphenols but the extension of maceration time at high temperatures causes a decrease in the amount of these compounds due to the degradation of anthocyanins and phenolic acids. The phenolic acids showed different sensitivities regarding high temperatures. Chromatographic analysis revealed that malvidin-3-O-glucoside was the major anthocyanin monomer detected whereas cyanidin-3-O-glucoside was the minor anthocyanins; also, these analyses showed that epigallocatechin (monomeric tannin) was the most representative of flavan-3-ols.

In addition to maceration temperature and time, differences illustrated during the maceration are due also to the effect of terroir and vintage. Syrah Florentine showed higher total polyphenols concentrations than Syrah Saint Thomas suggesting that the accumulation of phenolic compounds in grape berries is strongly affected by „‟terroir‟‟‟ factors. The terroir effect for Cabernet Sauvignon musts was less important than those of Syrah musts, in fact for this variety higher maceration

temperatures masked terroir effects. Results showed also that Syrah Florentine was the most suitable terroir for obtaining stilbene-enriched wines. Vintage effect was observed on each studied phenolic compound concentration and was more important for Syrah than for Cabernet Sauvignon. 2014 vintage for both grape varieties exhibited higher phenolic content comparing to 2015 and this can be due to some particular weather conditions.

The addition of maceration enzymes to the macerated musts promoted higher concentration of anthocyanins (TA), phenolic compounds (TP), tannins (T) as well as higher values of color intensity (CI) and polyphenol index (TPI) and different HPLC phenolic profiles compared to those macerated at the same temperature without added enzymes. The antioxidant activity (ABTS) was also higher probably due the higher polyphenolic content.

After alcoholic fermentation, an increase of some flavanols and non flavanols (catechin, epicatechin, procyanidin B1 and B2 and gallic acid contents in wines fermented by the two yeast strains (X and Y) is observed which is probably the consequence of the hydrolysis that suffer their polymeric and galloylated precursors during alcoholic fermentation. After alcoholic fermentation an increase of free caffeic and ferulic acids contents in wines fermented by the two yeast strains resulted from the hydrolysis of both caffeic and ferulic tartaric acid esters. Wines fermented by Y strain showed a significantly higher anthocyanin content than the wines fermented by X strain while X strain revealed higher content of total non-anthocyanin compounds especially gallic acid. Wines fermented by X strain showed significantly increment in the content of trans-resveratrol while the trans-resveratrol content was significantly decreased in wines fermented by Y strain. Results could be explained by a higher β-glucosidase activity of the X strain and different adsorption characteristics of its cell wall.

After alcoholic fermentation, discriminant analyses showed that Syrah and Cabernet Sauvignon Saint Thomas wines were mainly separated according to yeast strains and glycosylated delphinidin was the anthocyanin the most affected by the yeast strain. For Florentine wines, discriminant analyses showed that Syrah and Cabernet Sauvignon wines were mainly separated according to the grape varieties and procyanidin B2 was the variable with the highest discriminant power.

For 2014 vintage, discriminant analyses applied to Cabernet Sauvignon from the two different regions after alcoholic fermentation showed that that wine samples were mostly discriminated according to the yeast strains; therefore, the yeast strain effects were maintained even when using grapes from the same variety but from different terroirs. On the other hand, the behavior of the two yeast strains varies depending on the temperature of the pre-macerated must, the origin of the grapes (two different terroirs) and vintages (2014 and 2015).

Concerning the fining agents, all treated wines with different fining agents showed a decrease in the content of all polyphenol except wines added by exogenous tannins. The decreased intensity is directly related to the type and the concentration of fining agent. Bentonite had the highest impact on the anthocyanins contents of wines followed by oenological tannins, whereas, Pvpp + casein and mannoproteins decreased significantly tannin levels. The addition of oenological tannins increases total tannins while it decreases significantly the total anthocyanins. Vegetable proteins and gelatin showed the lowest impact on the wine phenolic composition. Epigallocatechin was the principal phenolic removed by bentonite treatment. PVPP + casein showed to mainly remove catechin and epigallocatechin. Procyanidins B1 and B2 were the flavanols mainly removed by gelatin and egg albumin. All wine treated with fining agents didn‟t show any effect on the phenolic acids and resveratrol contents in wine samples. Results revealed the importance of using the recommended minimum amount of all fining agents in order to have high phenolic composition.

After each winemaking step, the biological activities were measured. Results for maceration step showed that Syrah Saint Thomas macerated at 70°C for 48 hours exhibited higher biological activities studied compared to Syrah-Florentine macerated at the same temperature (althought that this latter showed higher phenolic compounds than Sy-St after maceration). Syrah Saint Thomas control exhibited higher antidiabetic activities than Syrah-Saint Thomas macerated at 70°C for 48 and 24 hours respectively for the 2014 and 2015 vintage. Cabernet Sauvignon Saint Thomas control showed higher anti-inflammatory and antidiabetic activities than Cabernet Sauvignon Saint Thomas macerated at 70°C for 48 and 24 hours respectively for 2014 and 2015 vintages. Biological activities analyses of musts showed that higher antidiabetic and anti-inflammatory activities were more correlated to the high anthocyanin and phenolic acid content. After alcoholic fermentation (with few exceptions of some antioxidant activities), almost all of the wine samples presented an increase with

the emergence of new types of biological activities which doesn‟t existed at must level. The control with added enzymes (25°C-Y + enzymes) had the highest percentage of inhibition for all of the biological activities studied, on which anti-LOX and anti-α-glucosidase showed respectively maximum activity. The antioxidant activity of the final product depends on the qualitative and quantitative composition of polyphenols. The antioxidant activity was little affected by fining agents except the addition of condensed tannins that increased it. Antiradical activities of wines treated with fining agents were more correlated with the flavan-3-ol fraction than the anthocyanins.

The perspectives that emerge from this work can be directed as follows:

- Extend the study on the different Lebanese red grape varieties to generalize the results obtained with Syrah and Cabernet sauvignon varieties

- Extend the study on the different Lebanese terroirs and vintages on the content of polyphenols

- Reproduce the experiments on an industrial scale to confirm the results and findings obtained

- Establish a link between the biological activities and the compounds responsible.

- Study the impact of different commercial yeast strains used in the Lebanese wine industry on the phenolic composition of wines

- The completion of this study by revealing the wine aromas through analyzing wines by gas chromatography coupled to mass spectrophotometry and by making sensory evaluation

- Development of the thiolysis method in order to define the mean degree of polymerization, the content and the type of proanthocyanidins.

- Completing the impact of each winemaking step by studying the impact of ageing in tanks and in oak barrels.

Annexes

ANNEXE I: Effect of malolactic fermentation on the phenolic composition and biological activities of wines

This part "effect of malolactic fermentation on the phenolic composition and biological activities of wines"""has been set up under Annexe I for two reasons. First, because X strain does not allow the set off of malolactic fermentation (MLF) and second, due to the long duration of MLF (nearly 2 months), we recorded an oxidation of phenolic compounds since manipulations were carried out under laboratory conditions (high oxygen diffuses).

I.1. Materials and methods

I.1.1. Chemicals, culture media and standards (see II.2.1. p. 161)

I.1.2. Strains and storage conditions

Oenococcus oeni Z strain used in this work were kindly provided by Lallemand Inc. (Blagnac, France). The bacterial strain was kept frozen at −20°C in MRS (De Man, Rogosa and Sharpe) broth containing 20% glycerol (v/v).

I.1.3. Vinifications

After completion of AF, the fermented musts from two vintages (2014 and 2015), two grape varieties (Syrah and Cabernet Sauvignon) and two distinct regions (Florentine and Saint Thomas) using either X or Y strain were subjected to different steps before inoculation of the lactic acid bacteria. First yeast cells were removed by centrifugation (3000 rpm for 20 min at 4°C) and the supernatants were recovered. Then, the L-malic acid concentration was measured and readjusted to 3 g/l (enzymatic assay, Boehringer Mannheim/R-Biopharm, kit. No 10139068035, Darmstadt-Germany). Next, the pH was adjusted to 3.5 using a 10 mol/l NaOH solution. Finally, the wines were filtered aseptically through 0.22 µm membranes (Elvetec services) and were inoculated with the malolactic bacteria at an initial concentration of 2×10^6 cells/ml (Petroff-Hausser counting chamber). The MLF was followed until cessation of L-malic acid consumption. The bacterial inoculum was prepared in two steps. First, a preculture of *Oenococcus oeni* Z strain was obtained by reactivating the stock culture in MRS broth composed of 10 g/l Peptone, 8 g/l Meat extract, 4 g/l Yeast extract, 20 g/l D (+) – Glucose, 2 g/l Dipotassium hydrogen phosphate, 5 g/l Sodium acetate

trihydrate, 2 g/l Triammonium citrate, 0.2 g/l Magnesium sulfate heptahydrate, 0.05 g/l Manganous sulfate heptahydrate with 3% ethanol (v/v) added. After 24 h, the preculture was used to inoculate the low sugar concentration synthetic grape juice medium composed of 50 g/l D-Glucose, 1 g/l Yeast extract, 2 g/l Ammonium sulfate, 0.3 g/l Citric acid, 5g/l L-malic acid, 5 g/l L-tartaric acid, 0.4 g/l Magnesium sulfate, 5 g/l Potassium dihydrogen phosphate with 6% ethanol (v/v) added. This step provided the bacterial inoculum after an incubation period of 24 h. All fermentation steps were carried out at 22°C, with stirring at 150 rpm in Erlenmeyer flasks and involved 80 ml volumes of wine. Phenolic compounds were analysed at the end of malolactic fermentation (60 days for must fermented by Y strain). Wine samples were stored at 2°C until analyzed. All fermentations were performed in duplicate.

I.1.4. Spectrophotometric determinations (see II.1.2.5. p. 88)

I.1.5. HPLC analysis of phenolic compounds (see II.1.2.6. p. 89)

I.1.6. Determination of Biological Activities (see II.1.2.7. p. 89-93)

I.2. Results and discussions

I.2.1. Evolution of phenolic compounds after MLF

Table A.I.1, A.I.2, A.I.3, A.I.4, A.I.5, A.I.6, A.I.7, A.I.8 and A.I.9 showed the spectrophotometric and HPLC determination (mg/l) of phenolic compounds in wines fermented by Y yeast strain before and after malolactic fermentation from Syrah and Cabernet Sauvignon from two consecutive vintages and two distinct regions. As observed (Table A.I.1, A.I.2, A.I.3, A.I.4, A.I.5, A.I.6, A.I.7, A.I.8 and A.I.9), after malolactic fermentation, all wine samples indicated large decrease in the concentration of total and individual polyphenols associated with their oxidations under laboratory conditions (high oxygen diffuses). Only Y fermented wines induce malolactic fermentation whereas X strain does not allow the start off MLF. These Results were in accordance with those of Rizk et al., 2016 who showed that the antibacterial proteinaceous metabolites produced by X strain inhibit the malolactic enzyme activity of *Oenococcus oeni* Z strain and consequently no demalication was detected.

Table A-I.1: Spectrophotometric determination of total anthocyanin, phenolic profile, and antioxidant activity in wines (mg/l) from *Vitis vinifera* L. cv. Syrah and Cabernet Sauvignon Saint Thomas of 2014 vintage, before and after malolactic fermentation (MLF) resulting from wine premacerated at different temperatures (10°C, 60°C, 70°C and 80°C) and fermented by Y yeast strain

		Sy-ST-2014		CS-ST-2014	
		Before MLF	After MLF	Before MLF	After MLF
10°C	TA	50.75± 3.71	9.62 ± 1.24	85.31 ± 0.62	31.50 ± 0.00
	TPI	7.8 ± 0.14	4.55 ± 0.14	13.00 ± 0.00	9.25 ± 0.07
	TP	230.00 ± 7.07	165.00 ± 7.03	487.50 ± 3.53	265.00 ± 0.00
	T	869.85 ± 27.34	77.32 ± 0.00	425.26 ± 0.00	299.61 ± 13.66
	ABTS	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
60°C	TA	72.62 ± 4.95	28.87 ± 0.00	182.87 ± 1.24	51.19 ± 0.62
	TPI	32.85 ± 0.07	28.50 ± 0.70	39.65 ± 1.06	30.05 ± 0.07
	TP	1567.50 ± 31.82	1117.50 ± 3.52	1992.50 ± 3.53	967.50 ± 3.55
	T	705.54 ± 13.67	386.60 ± 0.00	2628.88 ± 27.34	1198.46 ± 0.00
	ABTS	4.35 ± 0.07	4.50 ± 0.00	4.40 ± 0.00	4.00 ± 0.01
70°C	TA	78.75± 1.42	17.94 ± 0.62	187.25 ± 3.71	42.44 ± 0.62
	TPI	41.35 ± 0.07	38.35 ± 0.07	52.45 ± 0.49	37.85 ± 0.07
	TP	2265 ± 21.21	1332.50 ± 3.53	2755 ± 14.14	1765.00 ± 7.08
	T	2725.53 ± 0.00	1749.36 ± 68.34	3566.38 ± 13.67	1933.00 ± 0.00
	ABTS	3.70 ± 0.00	3.35 ± 0.07	3.15 ± 0.21	4.15 ± 0.07
80°C	TA	61.25± 0.00	14.00 ± 1.24	72.62 ± 0.00	25.37 ± 2.47
	TPI	38.90 ± 0.08	41.55 ± 0.21	41.30 ± 1.41	53.40 ± 1.98
	TP	1720.00 ± 0.00	1577.50 ± 10.60	2052.50 ± 3.54	2020.00 ± 0.00
	T	2725.53 ± 136.68	1556.05 ± 13.67	2638.54 ± 12.20	1923.33 ± 13.67
	ABTS	3.70 ± 0.00	4.15 ± 0.42	4.65 ± 0.50	4.20 ± 0.00

Mean (n =2) ± SD. TA, total anthocyanins; TPI, total phenolic index; TP, total phenolics; T, Tannins; Sy-ST, Syrah Saint Thomas; CS-ST, Cabernet Sauvignon Saint Thomas.

Table A-I.2: Spectrophotometric determination of total anthocyanin, phenolic profile, and antioxidant activity in wines (mg/l) from *Vitis vinifera* L. cv. Syrah and Cabernet Sauvignon Florentine of 2014 vintage, before and after malolactic fermentation (MLF) resulting from wine premacerated at different temperatures (10°C, 60°C and 70°C) and fermented by Y yeast strain.

		Sy-F-2014		CS-F-2014	
		Before MLF	**After MLF**	**Before MLF**	**After MLF**
10°C	TA	34.56 ± 0.08	12.69 ± 0.62	45.06 ± 5.57	28.87 ± 2.47
	TPI	7.40 ± 0.14	11.75 ± 0.91	9.10 ± 0.71	12.00 ± 0.42
	TP	302.50 ± 10.60	135.00 ± 14.14	362.50 ± 3.53	207.50 ± 10.61
	T	106.31 ± 13.66	57.99 ± 0.00	309.28 ± 0.00	260.95 ± 0.15
	ABTS	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
60°C	TA	99.31 ± 1.86	54.69 ± 0.00	260.31 ± 5.42	125.56 ± 0.62
	TPI	42.90 ± 0.56	39.60 ± 0.14	41.80 ± 0.34	34.15 ± 2.33
	TP	1955.00 ± 7.07	1487.50 ± 3.53	2050.00 ± 0.00	1287.50 ± 3.53
	T	2271.27 ± 13.67	1643.05 ± 27.23	2203.60 ± 0.00	956.83 ± 13.66
	ABTS	4.50 ± 0.21	4.60 ± 0.12	4.40 ± 0.01	4.50 ± 0.02
70°C	TA	38.06 ± 4.33	29.75 ± 5.56	102.37 ± 4.95	56.44 ± 0.62
	TPI	54.90 ± 5.23	43.35 ± 1.76	54.80 ± 2.26	40.50 ± 0.00
	TP	2262.50 ± 17.68	1865.00 ± 21.21	2492.50 ± 3.25	1402.50 ± 3.31
	T	2580.55 ± 68.34	2329. 26± 95.68	3170.12 ± 2.14	1836.35± 1.20
	ABTS	4.00 ± 0.14	4.25 ± 0.21	4.05 ± 0.02	3.65 ± 0.00

Mean (n =2) ± SD. TA, total anthocyanins; TPI, total phenolic index, TP, total phenolics; T, Tannins; Sy-F, Syrah Florentine; CS-F, Cabernet Sauvignon Florentine

Table A-I.3: Anthocyanin profiles (mg/l) in wines from *Vitis vinifera* L. cv. Syrah and Cabernet Sauvignon Saint Thomas of 2014 vintage, before and after malolactic fermentation (MLF) resulting from wine premacerated at different temperatures (10°C, 60°C, 70°C and 80°C) and fermented by Y yeast strain

		Sy-ST-2014		CS-ST-2014	
		Before MLF	After MLF	Before MLF	After MLF
10°C	Dp	2.53 ± 0.47	1.71 ± 0.00	2.94 ± 0.00	2.08 ± 0.01
	cy	n.d	n.d	1.64 ± 0.00	n.d
	pn	n.d	n.d	n.d	n.d
	Mv	5.97 ± 0.07	n.d	8.43 ± 1.25	n.d
60°C	Dp	5.32 ± 0.63	1.93 ± 0.02	13.12 ± 2.62	4.00 ± 0.00
	cy	1.43 ± 1.44	n.d	1.75 ± 0.03	n.d
	pn	1.00 ± 0.04	n.d	0.81 ± 0.08	n.d
	Mv	9.52 ± 9.52	n.d	19.50 ± 4.25	n.d
70°C	Dp	5.93 ± 0.57	1.96 ± 0.02	12.25 ± 1.79	2.63 ± 0.02
	cy	n.d	n.d	n.d	n.d
	pn	n.d	n.d	n.d	n.d
	Mv	2.73 ± 0.04	n.d	4.28 ± 0.04	n.d
80°C	Dp	4.73 ± 0.80	1.92 ± 0.03	5.59 ± 0.05	2.21 ± 0.00
	cy	n.d	n.d	n.d	n.d
	pn	n.d	n.d	n.d	n.d
	Mv	n.d	n.d	n.d	n.d

Mean (n =2) ± SD. Dp, delphinidin-3-O-glucoside; Cy, cyanidin-3-O-glucoside; Pn, peonidin-3-O-glucoside; Mv, malvidin-3-O-glucoside; n.d, not detected values; Sy-ST, Syrah Saint Thomas; CS-ST, Cabernet Sauvignon Saint Thomas.

Table A-I.4: Anthocyanin profiles (mg/l) in wines from *Vitis vinifera* L. cv. Syrah and Cabernet Sauvignon Florentine of 2014 vintage, before and after malolactic fermentation (MLF) resulting from wine premacerated at different temperatures (10°C, 60°C and 70°C) and fermented by Y yeast strain

		Sy-F-2014		CS-F-2014	
		Before MLF	After MLF	Before MLF	After MLF
10°C	Dp	2.78 ± 0.09	1.97 ± 0.00	2.91 ± 0.05	2.95 ± 0.02
	cy	n.d	n.d	2.14 ± 0.01	1.94 ± 0.02
	pn	0.76 ± 0.01	n.d	0.87 ± 0.01	n.d
	Mv	6.89 ± 0.65	n.d	10.29 ± 0.27	n.d
60°C	Dp	8.35 ± 0.06	3.60 ± 0.00	16.39 ± 0.43	9.18 ± 0.68
	cy	n.d	n.d	1.94 ± 0.03	n.d
	pn	0.74 ± 0.00	n.d	1.06 ± 0.08	n.d
	Mv	3.58 ± 0.39	n.d	22.59 ± 1.05	n.d
70°C	Dp	7.50 ± 0.40	3.59 ± 0.01	12.66 ± 0.97	6.21 ± 0.87
	cy	n.d	n.d	n.d	n.d
	pn	n.d	n.d	n.d	n.d
	Mv	n.d	n.d	4.64 ± 0.04	n.d

Mean (n =2) ± SD. Dp, delphinidin-3-O-glucoside; Cy, cyanidin-3-O-glucoside; Pn, peonidin-3-O-glucoside; Mv, malvidin-3-O-glucoside; n.d, not detected values; Sy-F, Syrah Florentine; CS-F, Cabernet Sauvignon Florentine

Table A-I.5: Flavan-3-ols profiles (mg/l) in wines from *Vitis vinifera* L. cv. Syrah and Cabernet Sauvignon Saint Thomas of 2014 vintage, before and after malolactic fermentation (MLF) resulting from wine premacerated at different temperatures (10°C, 60°C, 70°C and 80°C) and fermented by Y yeast strain

		Sy-ST-2014		CS-ST-2014	
		Before MLF	After MLF	Before MLF	After MLF
	Cat	18.46 ± 1.27	2.36 ± 0.06	24.25 ± 1.58	2.90 ± 1.09
	Epi	23.94 ± 2.23	17.03 ± 0.02	22.78 ± 0.01	17.57 ± 0.63
	EpiG	25.77 ± 3.26	11.45 ± 0.00	40.91 ± 1.22	23.63 ± 0.54
	Epig	2.44 ± 0.22	1.95 ± 0.01	3.09 ± 0.83	2.76 ± 0.10
	pro B1	10.31 ± 0.82	2.14 ± 0.04	12.41 ± 0.33	4.37 ± 1.83
	Pro B2	13.35 ± 2.57	15.84 ± 0.05	6.29 ± 0.61	3.24 ± 0.96
10°C	G.A	0.24 ± 0.11	3.80 ± 0.00	9.68 ± 0.08	8.61 ± 1.14
	C.A	1.63 ± 0.02	1.34 ± 0.00	1.78 ± 0.20	1.45 ± 0.21
	F.A	1.83 ± 6.68	1.13 ± 0.90	3.06 ± 0.37	3.25 ± 0.05
	Res	1.15 ± 0.62	0.80 ± 0.04	1.65 ± 1.33	2.17 ± 0.97
	Cat	69.47 ± 1.28	4.47 ± 0.39	68.00 ± 1.69	6.60 ± 0.02
	Epi	77.64 ± 1.25	37.13 ± 2.13	66.59 ± 2.86	53.63 ± 1.86
	EpiG	106.44 ± 0.70	14.22 ± 0.03	83.67 ± 1.41	21.47 ± 1.27
	Epig	17.71 ± 0.30	7.97 ± 1.10	15.66 ± 1.67	11.36 ± 1.56
60°C	pro B1	37.49 ± 2.47	155.13 ± 0.18	33.87 ± 0.22	151.75 ± 2.14
	Pro B2	120.71 ± 0.26	44.02 ± 0.06	120.95 ± 0.71	56.90 ± 2.34
	G.A	3.49 ± 0.12	17.42 ± 0.00	0.94 ± 0.19	27.00 ± 2.37
	C.A	5.50 ± 0.25	3.00 ± 0.00	3.09 ± 0.19	3.17 ± 0.07
	F.A	9.57 ± 0.26	3.17 ± 0.02	2.78 ± 0.87	6.41 ± 0.25
	Res	4.10 ± 0.04	0.95 ± 0.01	5.28 ± 0.14	2.63 ± 0.43
	Cat	69.59 ± 2.31	2.80 ± 0.66	86.35 ± 2.41	8.37 ± 0.00
	Epi	83.41 ± 2.85	37.33 ± 3.42	61.35 ± 1.35	44.44 ± 2.95
	EpiG	159.45 ± 1.43	10.52 ± 2.62	157.68 ± 0.49	78.27 ± 0.54
	Epig	24.41 ± 3.17	2.18 ± 0.38	30.92 ± 8.62	11.63 ± 0.50
70°C	pro B1	41.29 ± 2.37	25.86 ± 2.55	44.83 ± 3.85	114.12 ± 2.22
	Pro B2	127.76 ± 0.21	40.34 ± 4.21	135.96 ± 2.86	71.59 ± 1.78
	G.A	6.60 ± 0.12	39.85 ± 0.00	2.37 ± 0.31	33.17 ± 0.81
	C.A	6.57 ± 1.78	4.49 ± 0.59	3.85 ± 1.02	3.70 ± 0.00
	F.A	7.21 ± 1.34	1.43 ± 0.28	11.88 ± 3.69	3.35 ± 0.01
	Res	3.14 ± 0.16	0.48 ± 0.02	5.76 ± 0.25	1.20 ± 0.00
	Cat	77.78 ± 0.77	19.99 ± 0.16	96.22 ± 0.03	14.14 ± 0.01
	Epi	150.63 ± 0.59	78.40 ± 0.92	117.59 ± 1.82	47.84 ± 0.87
	EpiG	153.62 ± 1.38	118. 93 ± 0.84	212.68 ± 0.51	126.064 ± 0.29
	Epig	22.40 ± 0.07	15.69 ± 2.05	30.04 ± 2.39	23.43 ± 0.25
80°C	pro B1	45.99 ± 0.51	98.32 ± 1.84	41.71 ± 5.33	91.53 ± 1.78
	Pro B2	153.89 ± 1.74	82.80 ± 0.96	128.68 ± 0.32	68.04 ± 0.41
	G.A	6.94 ± 0.07	23.41 ± 0.59	7.07 ± 0.72	30.17 ± 0.12
	C.A	8.13 ± 0.03	4.65 ± 0.45	6.30 ± 0.89	5.61 ± 0.52
	F.A	6.41 ± 0.02	3.92 ± 0.22	6.55 ± 0.21	4.61 ± 0.13
	Res	3.57 ± 0.01	2.75 ± 0.26	2.79 ± 0.38	1.35 ± 0.04

Mean (n =2) ± SD. Cat, catechin; Epi, epicatechin; Epig, epicatechin gallate; EpiG, epigallocatechin; Pro B1, procyanidin B1; Pro B2, procyanidin B2; G.A, gallic acid; F.A, ferulic acid; Res, resveratrol; n.d, not detected values; Sy-ST, Syrah Saint Thomas; CS-ST, Cabernet Sauvignon Saint Thomas.

Table A-I.6: Flavan-3-ols profiles (mg/l) in wines from *Vitis vinifera* L. cv. Syrah and Cabernet Sauvignon Florentine of 2014 vintage, before and after malolactic fermentation (MLF) resulting from wine premacerated at different temperatures (10°C, 60°C and 70°C) and fermented by Y yeast strain

		Sy-F-2014		CS-F-2014	
		Before MLF	After MLF	Before MLF	After MLF
10°C	Cat	15.66 ± 3.47	2.77 ± 0.62	21.15 ± 0.83	1.89 ± 0.09
	Epi	23.26 ± 3.31	20.68 ± 3.80	12.60 ± 0.32	13.12 ± 0.88
	EpiG	25.63 ± 2.55	9.10 ± 1.88	37.26 ± 1.05	4.66 ± 0.38
	Epig	3.58 ± 0.46	3.44 ± 0.54	9.64 ± 0.17	3.02 ± 0.28
	pro B1	9.49 ±0.68	4.43 ± 0.15	11.19 ± 0.03	6.58 ± 0.49
	Pro B2	3.14 ± 2.10	24.24 ± 3.03	13.08 ± 0.43	12.96 ± 0.95
	G.A	0.27 ± 0.10	4.74 ± 0.15	0.02 ± 0.00	4.72 ± 0.30
	C.A	2.21 ± 0.00	1.42 ± 0.13	1.74 ± 0.00	1.64 ± 0.05
	F.A	2.49 ± 0.16	1.68 ± 0.24	2.49 ± 0.32	0.97 ± 0.03
	Res	0.83 ± 0.02	0.81 ± 0.054	1.44 ± 0.30	0.79 ± 0.02
60°C	Cat	75.94 ± 0.61	4.46 ± 0.02	67.04 ± 1.88	5.64 ± 0.01
	Epi	93.87 ± 0.36	47.33 ± 0.02	132.50 ± 6.32	102.04 ± 0.40
	EpiG	112.76 ± 0.24	9.15 ± 0.71	130.75 ± 2.59	34.51 ± 0.90
	Epig	23.44 ± 1.20	16.85 ± 0.01	31.70 ± 4.27	22.57 ± 0.79
	pro B1	46.66 ± 0.67	186.44 ± 2.91	43.49 ± 0.68	160.41 ± 0.24
	Pro B2	88.84 ± 2.43	32.14 ± 0.79	117.34 ± 5.45	54.07 ± 0.25
	G.A	2.41 ± 0.10	2.66 ± 0.01	0.69 ± 0.11	28.64 ± 0.39
	C.A	4.55 ± 0.03	3.99 ± 0.01	3.49 ± 0.17	3.56 ± 0.44
	F.A	4.40 ± 0.39	1.57 ± 0.02	9.97 ± 0.85	13.62 ± 0.04
	Res	2.30 ± 0.23	0.41 ± 0.00	9.95 ± 0.97	6.51 ± 0.11
70°C	Cat	91.31 ± 2.71	19.93 ± 4.06	82.60 ± 1.05	75.61 ± 0.53
	Epi	86.31 ± 2.89	66.33 ± 5.07	63.12 ± 0.45	41.53 ± 0.07
	EpiG	170.06 ± 2.14	124.93 ± 1.01	172.46 ± 1.64	50.04 ± 0.25
	Epig	32.60 ± 3.05	23.00 ± 0.25	24.58 ± 1.75	20.29 ± 0.27
	pro B1	53.88 ± 0.38	50.21 ± 0.87	51.85 ± 0.18	143.46 ± 0.83
	Pro B2	148.67 ± 5.05	111.53 ± 1.30	132.25 ± 1.78	82.05 ± 1.04
	G.A	5.12 ± 0.17	47.08 ± 2.60	3.59 ± 0.13	40.42 ± 0.10
	C.A	9.74 ± 0.25	6.21 ± 2.06	5.79 ± 0.14	5.23 ± 0.00
	F.A	16.20 ± 1.85	4.93 ± 1.13	21.32 ± 1.14	9.66 ± 0.67
	Res	5.66 ± 0.10	3.59 ± 1.05	5.64 ± 0.51	2.84 ± 0.02

Mean (n =2) ± SD. Cat, catechin; Epi, epicatechin; Epig, epicatechin gallate; EpiG, epigallocatechin; Pro B1, procyanidin B1; Pro B2, procyanidin B2; G.A, gallic acid; F.A, ferulic acid; Res, resveratrol; n.d, not detected values; Sy-F, Syrah Florentine; CS-F, Cabernet Sauvignon Florentine.

Table A-I.7: Spectrophotometric determination of total anthocyanin, phenolic profile, and antioxidant activity in wines (mg/l) from *Vitis vinifera* L. cv. Syrah and Cabernet Sauvignon of 2015 vintage, before and after malolactic fermentation (MLF) resulting from wine premacerated at different temperatures with or without added enzymes (60°C, 70°C, 70°C + enzymes, 25°C and 25°C + enzymes), fermented by Y yeast strain

		Sy-ST-2015		CS-ST-2015	
		Before FML	**After FML**	**Before FML**	**After FML**
60°C	TA	66.208 ± 1.82	37.21 ± 0.00	99.75 ± 2.33	61.54 ± 1.33
	TPI	44.667 ± 0.10	28.93 ± 0.06	50.27 ± 0.06	51.43 ± 0.29
	TP	1860 ± 0.00	933.33 ± 2.87	1986.67 ± 2.89	1685.00 ± 8.63
	T	1166.24 ± 29.52	502.58 ± 11.16	1275.78 ± 2.27	902.07 ± 9.16
	ABTS	3.833 ± 0.06	7.23 ± 0.02	3.73 ± 0.15	5.53 ± 0.09
70°C	TA	51.042 ± 1.33	32.96 ± 0.50	87.21 ± 1.82	34.71 ± 1.33
	TPI	61.3 ± 0.17	71.27 ± 0.11	71.80 ± 0.62	79.17 ± 2.57
	TP	1956.67 ± 7.63	1491.67 ± 5.77	2851.67 ± 2.89	1833.33 ± 2.85
	T	1527.07 ± 0.00	1082.48 ± 0.00	2036.10 ± 11.16	1784.80 ± 22.12
	ABTS	2.70 ± 0.17	3.43 ± 0.06	2.47 ± 0.06	3.30 ± 0.035
70°C + enzymes	TA	76.71 ± 1.34	56.29 ± 0.50	93.92 ± 1.34	41.42 ± 2.02
	TPI	83.53 ± 0.38	161.83 ± 0.06	53.30 ± 0.10	56.47 ± 0.32
	TP	2686.67 ± 5.77	2011.67 ± 2.89	2736.67 ± 2.87	1856.67 ± 2.75
	T	1752.59 ± 10.23	1404.65 ± 9.41	1829.91 ± 8.45	1520.63 ± 7.29
	ABTS	2.35 ± 0.00	2.40 ± 0.00	3.13 ± 0.12	6.80 ± 0.06
Control-25°C	TA	66.21 ± 0.50	17.21 ± 1.01	82.83 ± 1.01	7.00 ± 0.00
	TPI	44.67 ± 0.30	28.93 ± 0.80	50.23 ± 0.64	30.97 ± 0.60
	TP	1860.00 ± 8.66	933.33 ± 7.63	2118.33 ± 2.89	961.67 ± 2.65
	T	1166.24 ± 3.16	502.58 ± 0.00	1295.10 ± 0.00	509.02 ± 1.86
	ABTS	3.83 ± 0.06	7.23 ± 0.30	3.37 ± 0.11	6.70 ± 0.11
Control-25°C + enzymes	TA	125.71 ± 3.53	19.83 ± 0.50	86.92 ± 1.34	25.08 ± 0.51
	TPI	48.43 ± 0.64	28.47 ± 0.21	53.10 ± 0.30	29.53 ± 0.58
	TP	2250.00 ± 0.00	1026.67 ± 1.23	2260.00 ± 0.00	890.00 ± 5.00
	T	1269.34 ± 22.32	599.23 ± 0.00	1346.66 ± 8.39	618.56 ± 0.00
	ABTS	5.00 ± 0.40	7.97 ± 0.43	3.40 ±0.15	6.50 ± 0.00

Mean (n =2) ± SD. TA, total anthocyanins; TPI, total phenolic index, TP, total phenolics; T, Tannins ; Sy-ST, Syrah Saint Thomas; CS-ST, Cabernet Sauvignon Saint Thomas

Table A-I.8: Anthocyanins profiles (mg/l) in wines from *Vitis vinifera* L. cv. Syrah and Cabernet Sauvignon Saint Thomas of 2015 vintage, before and after malolactic fermentation (MLF) resulting from wine premacerated at different temperatures with or without added enzymes (60°C, 70°C, 70°C + enzymes) compared to control (25°C and 25°C + enzymes) fermented by Y yeast strain

		Sy-ST-2015		CS-ST-2015	
		Before FML	After FML	Before FML	After FML
60°C	Dp	4.63 ± 0.04	2.53 ± 0.02	6.63 ± 0.15	4.95 ± 0.01
	cy	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	pn	0.83 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Mv	3.90 ± 0.04	0.00 ± 0.00	11.91 ± 0.13	1.25 ± 0.01
70°C	Dp	7.05 ± 0.11	0.37 ± 0.00	9.42 ± 0.25	0.63 ± 0.63
	cy	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	pn	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Mv	2.89 ± 0.01	0.00 ± 0.00	3.69 ± 0.03	0.00 ± 0.00
70°C + enzymes	Dp	7.05 ± 0.18	0.373 ± 0.37	10.01 ± 0.54	0.84 ± 0.03
	cy	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	pn	0.00 ± 0.00	0.00 ± 0.00	0.74 ± 0.00	0.00 ± 0.00
	Mv	2.89 ± 0.06	0.00 ± 0.00	5.98 ± 0.06	0.00 ± 0.00
Control-25°C	Dp	6.18 ± 0.11	0.52 ± 0.00	4.77 ± 0.78	0.46 ± 0.03
	cy	2.59 ± 0.06	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	pn	5.74 ± 0.12	0.00 ± 0.00	1.09 ± 0.04	0.00 ± 0.00
	Mv	57.82 ± 1.37	0.00 ± 0.00	26.17 ± 1.85	0.00 ± 0.00
Control-25°C + enzymes	Dp	5.53 ± 0.19	0.36 ± 0.03	4.82 ± 0.68	0.39 ± 0.04
	cy	2.29 ± 0.045	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	pn	3.39 ± 0.13	0.00 ± 0.00	1.18 ± 0.05	0.00 ± 0.00
	Mv	30.82 ± 1.21	0.00 ± 0.00	27.17 ± 1.42	0.00 ± 0.00

Mean (n =2) ± SD. Dp, delphinidin-3-O-glucoside; Cy, cyanidin-3-O-glucoside; Pn, peonidin-3-O-glucoside; Mv, malvidin-3-O-glucoside; n.d, not detected values; Sy-ST, Syrah Saint Thomas; CS-ST, Cabernet Sauvignon Saint Thomas

Table A-I.9: Flavan-3-ols profiles (mg/l) in wines from *Vitis vinifera* L. cv. Syrah and Cabernet Sauvignon Saint Thomas of 2015 vintage, before and after malolactic fermentation (MLF) resulting from wine premacerated at different temperatures with or without added enzymes (60°C, 70°C, 70°C + enzymes) compared to control (25°C and 25°C + enzymes) fermented by Y yeast strain

		SY-ST-2015		CS-ST-2015	
		Before FML	After FML	Before FML	After FML
60°C	Cat	26.78 ± 0.89	12.33 ± 0.01	27.37 ± 0.69	11.99 ± 0.44
	Epi	82.54 ± 1.31	72.81 ± 0.57	57.54 ± 0.98	49.21 ± 0.10
	EpiG	97.20 ± 0.91	4.09 ± 0.06	99.74 ± 4.07	8.28 ± 0.81
	Epig	8.29 ± 0.06	5.37 ± 0.00	10.46 ± 0.12	8.54 ± 0.39
	pro B1	32.92 ± 0.68	21.86 ± 0.46	45.28 ± 1.93	36.24 ± 0.64
	Pro B2	73.94 ± 1.03	32.45 ± 0.00	75.73 ± 1.12	39.20 ± 2.97
	G.A	32.80 ± 0.58	25.36 ± 0.01	28.99 ± 0.86	21.94 ± 2.23
	C.A	4.86 ± 0.07	4.95 ± 0.01	3.13 ± 0.02	3.66 ± 0.27
	F.A	37.45 ± 0.72	6.25 ± 0.01	39.09 ± 1.00	7.32 ± 0.35
	Res	6.30 ± 0.04	1.25 ± 0.00	8.15 ± 0.01	2.34 ± 0.02
70°C	Cat	28.71 ± 1.52	15.14 ± 0.56	41.69 ± 1.08	13.63 ± 0.35
	Epi	85.38 ± 2.00	68.81 ± 0.54	97.17 ± 1.61	51.56 ± 2.57
	EpiG	94.68 ± 5.84	26.83 ± 1.08	236.07 ± 3.88	30.90 ± 2.53
	Epig	20.14 ± 0.01	7.87 ± 0.02	22.06 ± 0.67	15.22 ± 0.08
	pro B1	42.61 ± 0.94	32.65 ± 0.14	65.05 ± 1.25	23.96 ± 2.58
	Pro B2	83.95 ± 0.03	37.84 ± 0.61	112.51 ± 0.84	38.47 ± 3.37
	G.A	46.87 ± 3.03	37.93 ± 0.15	48.18 ± 1.70	39.79 ± 0.97
	C.A	5.26 ± 0.17	5.77 ± 0.03	3.62 ± 0.06	3.54 ± 0.08
	F.A	33.35 ± 2.36	5.57 ± 0.02	55.49 ± 0.36	7.15 ± 0.33
	Res	13.45 ± 1.64	2.51 ± 0.08	5.29 ± 1.21	2.57 ± 0.33
70°C + enzymes	Cat	35.13 ± 0.25	15.38 ± 0.01	36.81 ± 1.20	13.19 ± 0.46
	Epi	47.29 ± 0.27	27.69 ± 0.14	74.31 ± 2.25	49.77 ± 0.92
	EpiG	43.26 ± 1.93	27.68 ± 0.02	154.16 ± 4.91	25.34 ± 2.62
	Epig	20.24 ± 0.02	8.83 ± 0.02	28.54 ± 0.10	2.14 ± 0.03
	pro B1	46.96 ± 1.59	45.60 ± 0.45	59.35 ± 1.69	45.27 ± 0.29
	Pro B2	256.39 ± 5.45	39.65 ± 0.00	95.64 ± 2.34	40.41 ± 1.73
	G.A	45.39 ± 2.28	44.06 ± 0.00	47.21 ± 0.56	36.47 ± 0.14
	C.A	5.56 ± 0.83	5.66 ± 0.00	3.06 ± 0.39	3.36 ± 0.04
	F.A	35.77 ± 0.95	6.26 ± 0.03	33.26 ± 3.97	7.63 ± 0.56
	Res	16.53 ± 0.27	2.54 ± 0.00	5.19 ± 0.11	2.38 ± 0.30
Control-25°C	Cat	37.20 ± 5.78	6.61 ± 1.20	36.70 ±1.67	8.01 ± 0.18
	Epi	65.94 ± 3.33	7.95 ± 0.43	43.74 ± 1.96	35.36 ± 2.75
	EpiG	75.27 ± 3.39	17.74 ± 2.27	58.47 ± 0.81	15.57 ± 1.49
	Epig	20.08 ± 5.16	7.90 ± 0.50	29.20 ± 3.75	12.25 ± 0.78
	pro B1	55.18 ± 8.88	28.61± 2.54	77.59 ± 0.46	31.44 ± 4.07
	Pro B2	64.67 ± 3.37	31.55 ± 1.84	37.55 ± 1.29	34.41 ± 1.45
	G.A	17.76 ± 1.25	38.10 ± 6.96	18.53 ± 1.66	13.67 ± 0.18
	C.A	3.41 ± 0.08	2.43 ± 0.10	2.33 ± 0.14	3.67 ± 0.05
	F.A	48.35 ± 2.74	4.57 ± 0.42	17.94 ± 0.33	5.55 ± 0.21
	Res	5.57 ± 0.00	2.15 ± 0.01	5.57 ± 0.34	2.72 ± 0.08
Control-25°C + enzymes	Cat	57.54 ± 1.97	6.29 ± 0.34	54.84 ± 3.72	8.15 ± 0.17
	Epi	99.36 ± 4.46	6.97 ± 1.59	93.39 ± 1.13	39.18 ± 3.72
	EpiG	89.95 ± 3.22	11.49 ± 1.16	24.88 ± 1.32	17.44 ± 0.14
	Epig	30.35 ± 0.25	5.24 ± 0.88	35.57 ± 1.85	6.39 ± 0.39
	pro B1	50.19 ± 2.31	22.13 ± 1.89	91.39 ± 6.73	24.88 ± 2.18
	Pro B2	78.00 ± 2.65	22.85 ± 0.76	74.91 ± 2.57	32.35 ± 1.56
	G.A	22.68 ± 1.49	18.53 ± 0.23	22.15 ± 1.05	18.60 ± 0.50
	C.A	3.35 ± 0.07	2.33 ± 0.02	2.45 ± 0.10	3.31 ± 0.10
	F.A	54.80 ± 2.52	4.40 ± 0.80	134.25 ± 2.38	4.99 ± 0.21
	Res	11.25 ± 0.01	1.39 ± 0.20	11.51 ± 1.46	2.34 ± 8.21

Mean (n =2) ± SD. Cat, catechin; Epi, epicatechin; Epig, epicatechin gallate; EpiG, epigallocatechin; Pro B1, procyanidin B1; Pro B2, procyanidin B2; G.A, gallic acid; F.A, ferulic acid; Res, resveratrol; n.d, not detected values; Sy-ST, Syrah Saint Thomas; CS-ST, Cabernet Sauvignon Saint Thomas.

I.3. Biological activities

Figure A.I.1 and A.I.2 showed the biological activities of Syrah and Cabernet sauvignon Saint Thomas of 2014 vintage of musts and wines after malolactic fermentation (MLF). With few exceptions, CS-ST (Figure A.I.2) exhibited after MLF, increasing percentage inhibition of certain biological activities already present at must level with the occurrence of new activities which doesn"t existed at must grade. Whereas, contradictory results were showed for Sy-ST (Figure A.I.1) (decreasing percentage inhibition of biological activities after MLF). In fact, the interpretation of the results can be difficult related to the oxidation process that took place during our MLF analyses.

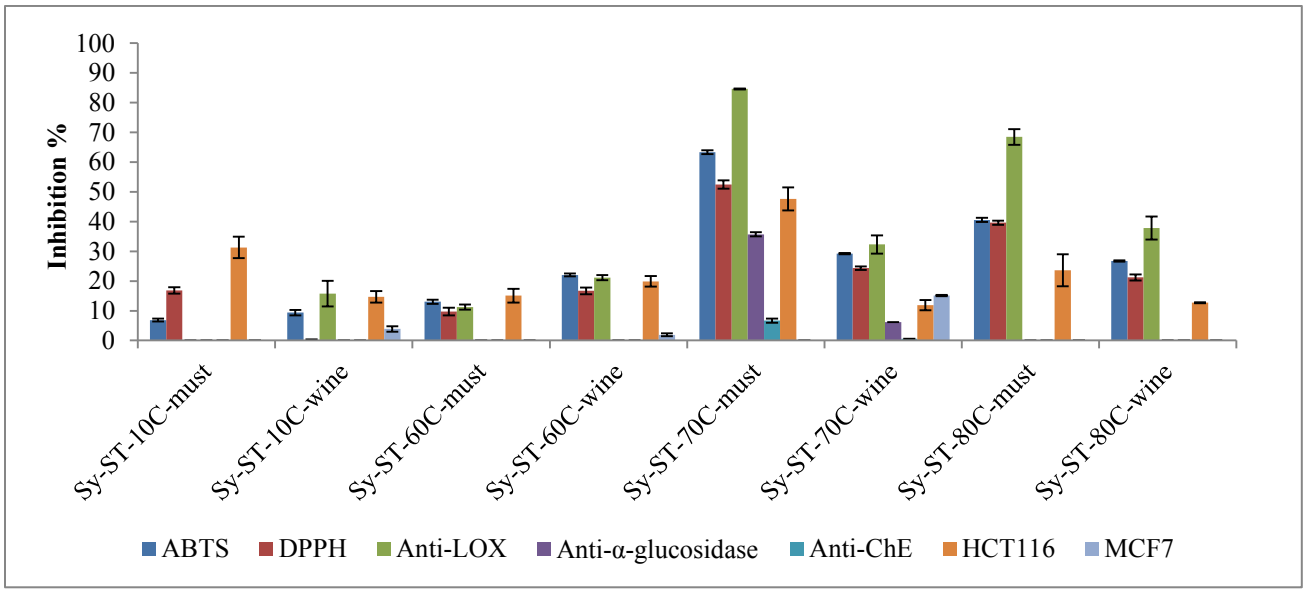

Figure A-I.1: Biological activities (ABTS, DPPH, Anti-LOX, Anti-α glucosidase, Anti-ChE, HCT116 and MCF7) of Sy-ST (Syrah Saint Thomas) musts and wines (after MLF accomplishement) premacerated at different temperatures (10°C, 60°C, 70°C, 80°C). Data were expressed as mean percentage inhibition (inhibition %) ± standard deviation

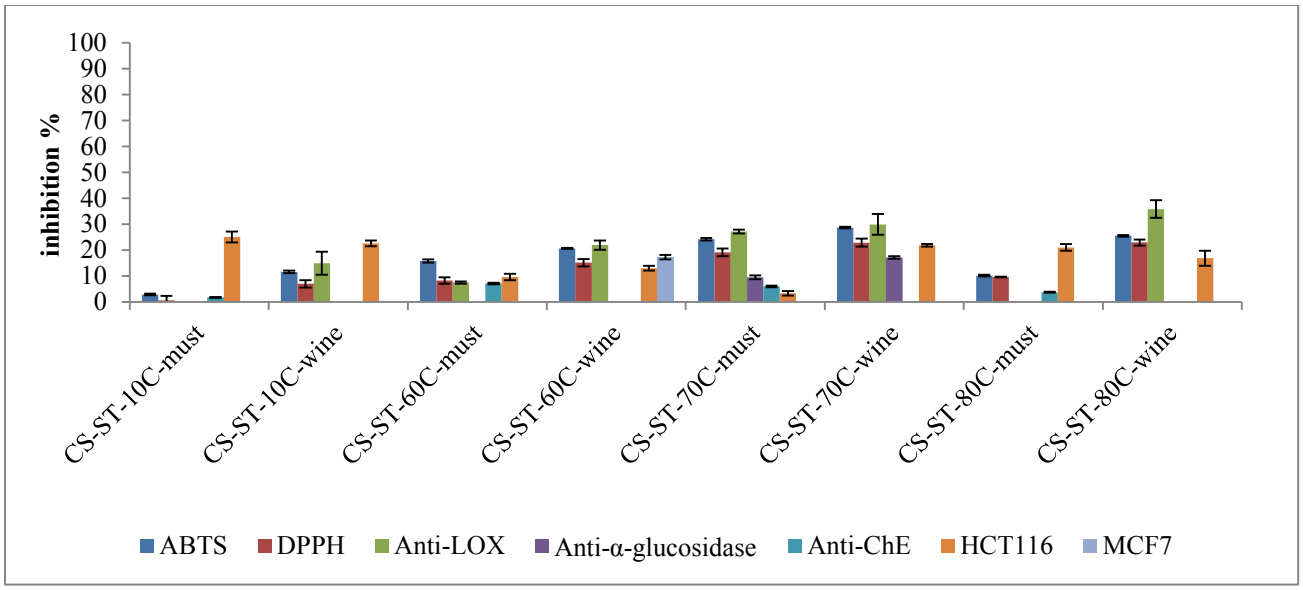

Figure A-I.2: Biological activities (ABTS, DPPH, Anti-LOX, Anti-α glucosidase, Anti-ChE, HCT116 and MCF7) of CS-ST (Cabernet Sauvignon Saint Thomas) musts and wine (after MLF) premacerated at different temperatures (10°C, 60°C, 70°C, 80°C) after 48 hours. Data were expressed as mean percentage inhibition (inhibition %) ± standard deviation.

ANNEXE II: Chromatograms

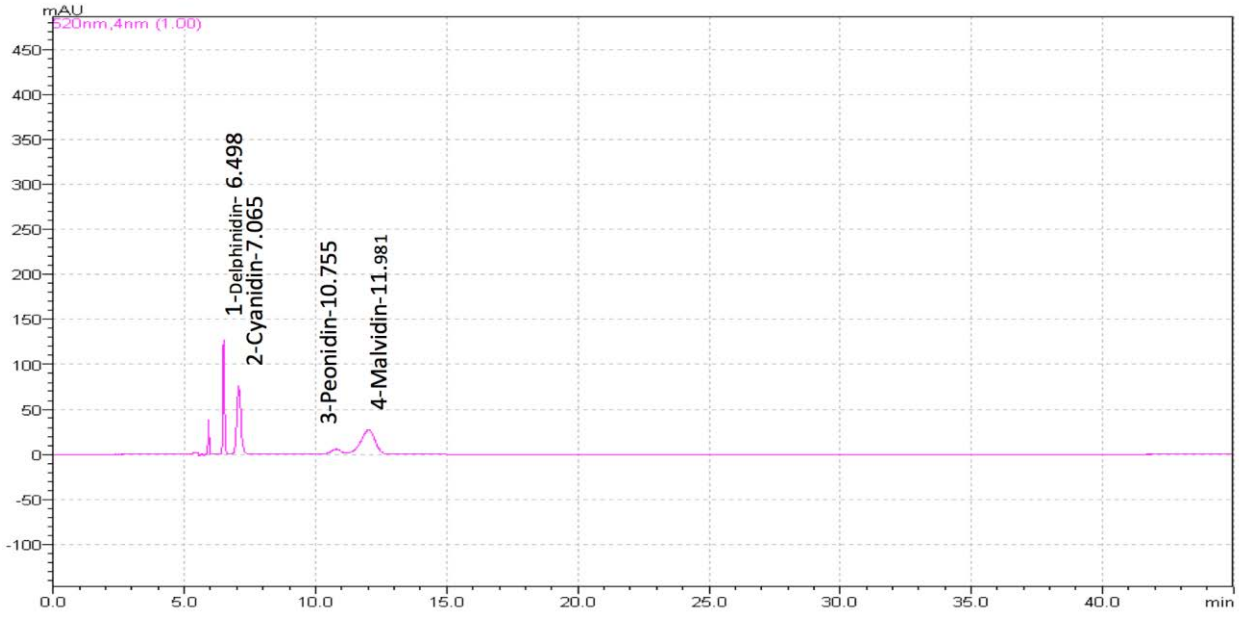

Figure A-II.1: HPLC Chromatogram of anthocyanins standards using UV–Vis detection at 520nm

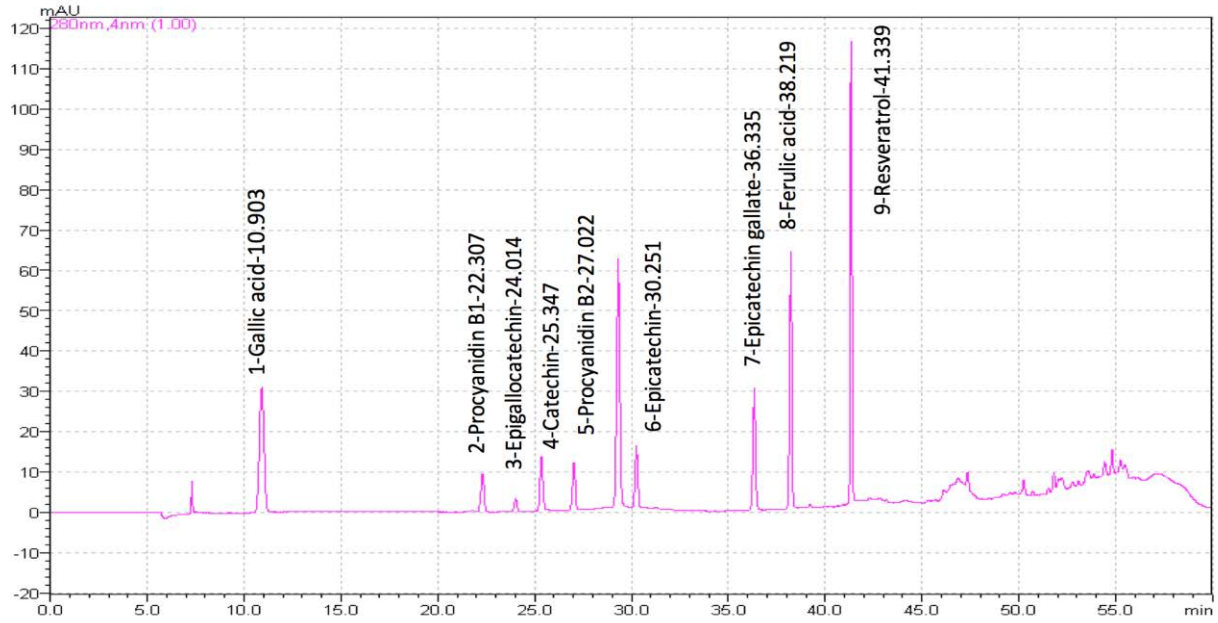

Figure A-II.2: HPLC Chromatogram of tannins standards using UV–Vis detection at 280nm

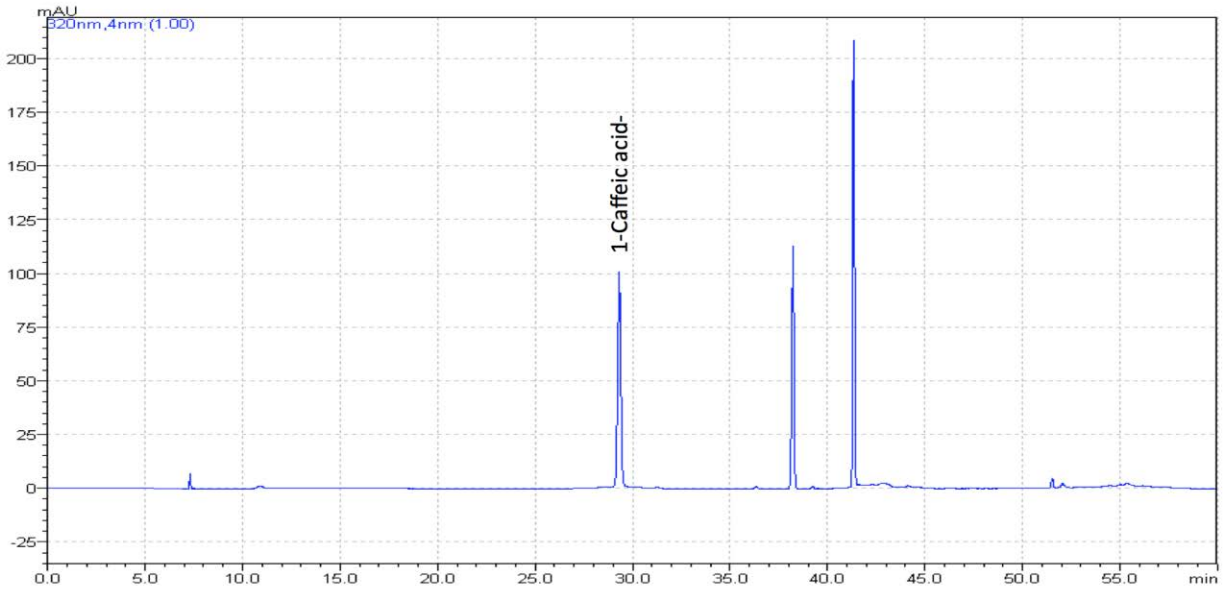

Figure A-II.3: HPLC Chromatogram of caffeic acid using UV–Vis detection at 320 nm

References

Rizk, Z., El Rayess, Y., Ghanem, C., Mathieu, F., Taillandier, P., Nehme, N. (2016). Involvement of proteinaceous compounds produced by a *Saccharomyces cerevisiae* strain in the inhibition of the growth and malolactic enzyme activity of an *Oenococcus oeni* strain. *International Journal of Food Microbiology*, 233, 90-96.